JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXI WASHINGTON, D. C., APRIL 15, 1921 NO. 2

SEED-COAT INJURY AND VIABILITY OF SEEDS OF WHEAT AND BARLEY AS FACTORS IN SUSCEPTIBILITY TO MOLDS AND FUNGICIDES [1]

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SEED-COAT INJURY AND VIABILITY OF SEEDS AS FACTORS IN SUSCEPTIBILITY TO SAPROPHYTIC FUNGI

Saprophytic fungi, infesting both soil and seed, constitute an important group of enemies to successful wheat and barley culture. The present investigation is (1) a study of some of the factors, especially the physical condition of the seed coat, which enable these saprophytes to attack the seed, and (2) the relation of mechanical injuries sustained by the seed coat to seed-treatment injury. The fungi used for the experimental infections were *Penicillium* sp. and *Rhizopus nigricans*. Penicillium causes serious losses where soil conditions favor its development and infection. Penicillium and Rhizopus are the two omnipresent molds which cause much trouble in blotter germinations in the laboratory. Rhizopus was not found attacking seeds in storage or in the soil.

It was noticed early in the study of germinating wheat in blotters that neither of these fungi was ever found on healthy, unbroken seeds, but that, if the seed coats were broken over the endosperm and germinated under nonsterile conditions, the seeds invariably were badly attacked. Neither fungus, however, attacked seeds whose only injury was a break in the coat over the embryo (Pl. 13).

Following these observations, experiments were planned to determine whether and under what conditions wheat seeds with unbroken coats or with the coat injured only over the embryo were immune from artificial infection with heavy inoculations of Penicillium and Rhizopus spores. The experiments were with hand-thrashed Early Baart wheat, injured with a dissecting needle and sown on blotters thickly sprinkled with

[1] This investigation was carried on in cooperation with the California Agricultural Experiment Station. The writer acknowledges with sincere gratitude the helpful suggestions of Profs. J. W. Gilmore and W. W. Mackie, of the University of California, and Dr. H. B. Humphrey, of the United States Department of Agriculture, during the progress of this investigation and the preparation of the report.

spores. The seeds were germinated in the laboratory in pans 8 inches square, covered with glass. Three different lots were always placed on the same blotter, one with uninjured seed coats, a second with seed coats broken by a scratch about 2 mm. long over the endosperm, and a third with seed coats broken over the tip of the radicle. The latter type of injury corresponds to the usual thrashing injury, the protruding tip being easily broken off in rough handling of the wheat. There usually were 50 seeds in each lot, but occasionally only 25.

After a few days, those seeds injured over the endosperm invariably showed masses of fungous hyphae growing from the crack in the seed coat. Only a small percentage of such seeds ever germinated, and none ever produced a healthy plant. When they did germinate, a deformed, spindling plumule might reach a length of a few centimeters before succumbing. The injury to the roots was less marked, though their development always was retarded. The other two lots of seed on the same blotter—that is, those with unbroken coats and those with coats broken over the embryo—invariably germinated and grew normally without a sign of fungous attack on either seed or seedling (Pl. 13; 14, A).

It seemed possible, however, that the apparently immune seeds might be susceptible if their germination was retarded, as occurs when seed is sown in cold, damp soil. To produce this retardation the experiments with Penicillium were repeated in the refrigerator at an average temperature of approximately 10° C. In all these experiments the germinators were removed from the refrigerator to the laboratory after seven days. When this was not done the fungi did not develop visibly, on account of the low temperature. After being brought to the warmer temperature of the laboratory the mycelium developed promptly within six days or less. Any seedling growth which had started was quickly stopped. This occurred most rapidly and fatally in the seeds broken over the endosperm, and least in the unbroken seeds. The deformity of the seedlings was extreme. The sheaths broke prematurely and the leaves were curled and wrinkled and were brown spotted, especially along the edges. The roots were short, few in number, devoid of root hairs, and brown tipped (Pl. 14, B).

It was found repeatedly in germinations in connection with other experiments that seeds which have been injured or killed by treatment with opper sulphate, or formaldehyde followed by drying, were easily attacked by Penicillium and Rhizopus regardless of the condition of the seed coats. After death the embryo, whether protected by an uninjured testa or exposed by a broken one, no longe had the immunity it had while living. Seeds which were weakened by treatment but which germinated well when sterilized with mercuric chlorid were covered with masses of the white, fluffy Rhizopus mycelium whenever germinated without previous disinfection. Therefore, along with the experiments to determine the relation of seed-coat injury to fungous attack, other

experiments were conducted to determine the relation of viability and vitality of the wheat to susceptibility to Penicillium and Rhizopus.

Perfect seeds were killed both by boiling for one minute, after which they were dried, and by prolonged exposure to a saturated solution of copper sulphate. The coats of some of these seeds were then broken over the embryo, some were broken over the endosperm, and others were left uninjured. Lots similarly treated were placed in the laboratory and in the refrigerator, those in the latter being removed after a week. Blotters sprinkled with spores of both Penicillium and Rhizopus were used. The results showed that, under all conditions of injury and of temperature, the dead seeds were attacked by both fungi, except that Rhizopus did not attack any seeds killed by copper sulphate. Penicillium is less sensitive to this chemical and therefore was much in evidence as green tufts of mycelium growing in the ruptures over both endosperm and embryo and spreading more thinly over the entire area of the unbroken seeds. Invariably, however, the latter were attacked more slowly and showed less abundant mycelium than those with seed coats broken. From this we conclude that the testa still was a considerable barrier to the invading hyphae.

Striking illustrations of the fact that seeds otherwise immune became very susceptible to attack by saprophytes when weakened or killed by treatment were afforded by many blotter germinations in connection with experiments on formaldehyde injury. It was found, in a series of lots of seed stored in atmospheres of progressively increasing humidity, that a point was reached where paraformaldehyde did not form upon the evaporation of formaldehyde, and at approximately that point the stored wheat ceased to show injury. The development of Rhizopus in the blotter germinations of these lots was found to furnish a good index of the amount of injury sustained by the samples, as it developed most quickly and luxuriantly on the worst injured samples, decreasing in amount until there was practically no mycelium on the uninjured seeds.

Experiments demonstrating that seed stored damp was uninjured by the 1 to 320 formaldehyde treatment,[1] while that allowed to dry under certain conditions suffered serious injury, showed this same relationship between viability and susceptibility to Rhizopus (*13*). The injured, dry-storage seeds were always more or less covered with Rhizopus mycelium, while the uninjured, damp-storage seeds remained clean (Pl. 19). The same results were obtained in experiments to show that washing with water after treatment removed the danger of formaldehyde injury on the drying of the seeds. The unwashed, injured seeds were severely attacked, while the washed, uninjured seeds were attacked but slightly, if at all (Pl. 20).

[1] The strength of formaldehyde solution used in seed treatment for smut is 1 pint of commercial formaldehyde in 40 gallons of water. The corresponding solutions used in this investigation consisted of 1 part of commercial formaldehyde in 320 parts. Since the commercial formaldehyde solution contained 36.2 per cent of formaldehyde gas, this dilution would be one part of formaldehyde in 884, or a 0.113 per cent solution.

The next step was to determine whether this close relation between the physical condition of the seed and susceptibility to fungous infection holds true of the more natural infections of seed germinating in the soil. Spores were obtained in quantity for soil infection by making cultures of Penicillium, isolated from wheat, in 500-cc. flasks containing crushed, autoclaved wheat as a medium. Variously injured lots of seed were prepared in the same way as those described for blotter germinations and were inoculated by soaking about 20 minutes in suspensions of Penicillium spores. The seeds were then sown in pots of sterile, sandy-loam soil into which had been mixed quantities of dry spores. After sowing, the seeds were watered with the spore suspension. Three sets of four pots each, consisting of infected endosperm-injured, embryo-injured, and uninjured seed and a control of sterile, uninjured seed were then placed in three different temperatures—namely, in the refrigerator, which averaged 10° C.; in the laboratory, at approximately 20°; and on an outer north window ledge having an intermediate temperature.

Because the growth of wheat is retarded considerably by heavy clay soils, a duplicate experiment was conducted in three sets of pots containing Yolo clay loam, in order that every possible chance for infection might be given.

When the seeds germinated, it was found that only two out of the six lots of endosperm-injured seed had escaped infection—those in the heavy clay loam in the refrigerator and those on the outer window ledge. Evidently infection was more difficult in this soil, possibly because of insufficient aeration. At the higher temperature of the laboratory, the seeds injured over the endosperm, when sown in this soil, produced only a low percentage of plants, and these were short and weak. The other pots of the clay-loam series, containing the infected, unbroken seeds and the radicle-injured seeds, produced plants which were as good as the control of uninfected seeds in height, development, and percentage of germination.

The same results were obtained from all three series of pots of the sandy loam, only those lots with seed coats broken over the endosperm being susceptible to Penicillium infection (Pl. 15). All these data are summarized in Table I.

TABLE I.—*Germination data from a typical experiment to determine the relation of seed-coat injury to susceptibility to Penicillium*

Nature of injury.	Germination.	Average height of seedlings.	Condition.
	Per cent.	*Cm.*	
Seed coats broken over embryo, infested.	96	15	Normal.
Seed coats broken over endosperm, infested.	84	4	Weak, with reduced root system; seeds covered with Penicillium.
Seed coats uninjured, infested..	100	15	Normal.
Control, uninjured, sterile.....	96	15	Do.

When the attacked seeds germinate, seedling growth is not always retarded, such plants occasionally coming up as soon as those of the other lots. After the plants reach a height of a few centimeters, however, growth practically stops. The plants are weak and often of a paler green than the uninjured seedlings, occasionally being somewhat yellowed. Actual deformity is not frequent, although the root systems often are stunted. When these seedlings are pulled up, the seed is found to be a mass of green mycelium. The uninjured seedlings are free from all evidences of fungous attack on the seed.

In a duplicate experiment to determine the relation of injured seed coats to susceptibility to invasion by Penicillium, no infection of even those seeds injured over the endosperm occurred where the temperature remained as low as 10° C. throughout. As was found in the blotter germinations, Penicillium requires a higher temperature for development. In this second experiment the injury to the susceptible seeds was very marked in those seedlings grown at the laboratory temperature of 20°, and distinct but less conspicuous at the intermediate temperature of the window ledge. Further study is needed to determine the optimum temperature for development and infection. These experiments indicate that the destruction of seed in the soil by Penicillium is dependent on poor physical condition or low vitality of the seed combined with temperature and moisture conditions favorable to the development of the fungus.

Besides attacking wheat in the soil, Penicillium is responsible for much injury to stored wheat which has not been thoroughly dried. To determine if this injury is also made possible by broken seed coats only, some hand-thrashed Early Baart seed was divided into three lots. The first was injured by means of a needle scratch over the endosperm, the second by a scratch over the embryo, and the third was left uninjured. Each lot was divided to make a duplicate set. All were soaked in a suspension of Penicillium spores for a few minutes and then put in closed bottles to keep them damp. Penicillium first appeared in the cuts of those injured over the endosperm. After two weeks the seed injured over the embryo became visibly infected at the point of injury, but at the end of three weeks the uninjured seed still showed no signs of fungous attack.

The experiment was repeated, using open vials as containers, which were kept in a damp chamber. Penicillium spores were dusted on the seeds until they were fairly green. In a few weeks green tufts of Penicillium were in evidence on the seed lots with the broken coats, but not until nearly two months had passed was there any sign of it on the unbroken seed. Only 40 per cent of these seeds with unbroken coats germinated at this time, although the original germination was 100 per cent. The other seeds were entirely killed (Pl. 18, B).

In this series of samples there were included some seeds which had been killed by boiling, then dried, inoculated with spores, and stored in the same way. Some of these were broken in the usual manner over the embryo. The latter were attacked quickly, much more so than the living seeds similarly injured. The dead seeds with unbroken coats were attacked later, but considerably more quickly than the living seeds with perfect coats (Pl. 18, B). The order in which the mycelium became evident on these different lots of seed is as follows:[1]

1. Seeds killed by boiling, seed coats broken over embryo.
2. Seeds living, seed coats broken over endosperm.
3. Seeds living, seed coats broken over embryo.
4. Seeds killed by boiling, seed coats unbroken.
5. Seeds living, seed coats unbroken.

The reason for the great susceptibility of wheat seed which has been scratched or broken over the endosperm is presumably clear. Rhizopus and Penicillium are saprophytes and grow readily in the exposed storage products of the endosperm. The embryo, however, is composed of living cells, and it is only under unusual conditions, if at all, that it can be directly attacked. Even in the few cases where it appeared to be directly infected, it is possible that the fungus reached the endosperm first and, developing there, killed the embryo by destroying its food or by poisoning it with the toxins produced. Entrance of the fungi into these seeds artificially injured over the endosperm results in death or injury to the embryo, probably by destroying the stored food rather than by actually entering the embryo itself. That this immunity of living embryos to direct attack is due to some property which disappears when death occurs is consistent with what we know of the behavior of plants in general toward saprophytes.

The practical importance of these facts is that the loss of seed through attack by soil saprophytes is due largely to one or two causes, both preventable: (1) The seed coats surrounding the endosperm are injured by improper handling (Pl. 17, B), or (2) the embryo has been injured by seed treatment with copper sulphate or other fungicide. In the first case, the embryo or seedling is unable to develop, probably because of the destruction of its food supply in the endosperm, and in the second case, it is so weakened by the chemical that its natural immunity from saprophytes is lost. Extreme instances of the first condition have occurred frequently in California. Wheat has been sold for seed which has been subjected to a scouring process preparatory to milling and was so badly scratched that it would not germinate well, even if untreated, so long as there were saprophytic fungi to infect it (Pl. 17). Such seed can not be germinated, even in sterile blotters, unless it is previously

[1] Seeds killed by boiling with seed coats broken over the endosperm were not included in these experiments because living seeds with endosperm injury are so very susceptible to fungous attack that it was deemed superfluous to try the effect of killing such seeds before breaking the seed coats.

disinfected (Pl. 18, A). When it is not sterilized and thus protected against infection, the seedling rarely even gets a start (Pl. 13). When to this chance of injury is added the effect of fungicides on the exposed embryos, there is more than sufficient reason for the failure of the plants from such seed to get above the surface of the ground.

HUMIDITY REQUIRED FOR THE GROWTH OF PENICILLIUM AND ASPERGILLUS ON STORED WHEAT

In an experiment to determine the relation of atmospheric humidity during storage to formaldehyde injury of wheat, Penicillium developed in the stored wheat in the three dampest samples only. The different humidities were produced in desiccators containing various mixtures of sulphuric acid and water, progressing by intervals of 10 per cent from a saturated atmosphere over water to one absolutely dry over concentrated sulphuric acid. The composition of the mixtures with water necessary to produce these different humidities was determined by Prof. C. W. Woodworth (*24*).[1]

Penicillium first appeared after 10 days on wheat stored in the saturated atmosphere and in that containing 90 per cent moisture. The mycelium continued to grow luxuriantly in these, and after 16 days it appeared in the one having 80 per cent humidity. In none of the drier lots did it ever develop, and it never was as abundant in the 80 per cent as in the 90 per cent and 100 per cent (saturated) chamber. Aspergillus was also very prevalent in the 80 per cent humidity but was largely overgrown in the other two atmospheres by the greater growth of Penicillium. In addition, Aspergillus developed to some extent in the desiccator with atmosphere containing 70 per cent moisture, indicating that its moisture requirements are less than those of Penicillium.

The humidities produced in the desiccators, and the development of Penicillium and Aspergillus in them, are shown in Table II.

TABLE II.—*Development of Penicillium and Aspergillus on wheat in atmospheres of different humidities*

Percentage of humidity.	Specific gravity of H_2SO_4 + H_2O mixtures.	Penicillium.[a]	Aspergillus.[a]
100	1.000	++	++
90	1.070	++	++
80	1.130	+	++
70	1.206	0	+
60	1.273	0	0
50	1.334	0	0
40	1.400	0	0
30	1.470	0	0
20	1.530	0	0
10	1.604	0	0
0	1.840	0	0

[a] The symbols used indicate that the fungus is abundant (++), fairly abundant (+), or lacking (0).

[1] Reference is made by number (italic) to "Literature cited," pp. 120-122.

EFFECT OF COPPER SULPHATE AND FORMALDEHYDE ON MOLD INFESTATION OF WHEAT IN STORAGE AND IN BLOTTER GERMINATIONS

The observations which follow were made for the most part in connection with experiments on seed-treatment injury; but, as they are sufficient to show certain facts very conclusively, it seems worth while to report them here.

Formaldehyde of the strength recommended for smut disinfection—1 part of commercial formaldehyde solution in 320—is also an effective fungicide for the spores of Rhizopus and Penicillium. The latter is especially sensitive to it. When seeds with scratched testas dusted with Rhizopus spores were dipped into a 1 to 320 solution for 10 minutes and sown on sterile blotters, they grew almost without infection. The controls which were not treated and were germinated on the same blotter were so badly attacked that very few seedlings developed, and the sample was quickly overgrown with masses of fluffy white mycelium.

It has been maintained by some that treatment with formaldehyde solutions renders wheat and barley more susceptible to subsequent fungous attack. Our own experiments failed to confirm such an assertion but disclosed a possible explanation of its origin. Whenever seed was injured or killed by being stored dry following formaldehyde treatment, it was very badly infected with Rhizopus if sown on a nonsterile blotter. Seeds treated in the same way but remaining uninjured because not allowed to dry germinated in the same blotters practically free from infection (Pl. 19). In germinations of treated wheat and barley stored in atmospheres of different humidities, Rhizopus appeared only on those samples which were injured, and even those from the damper atmospheres escaped infection almost entirely. Also, in experiments to show that washing with water removes the cause of injury upon drying, the same fact was shown by the invariable appearance of Rhizopus on the unwashed injured seeds and its absence on the washed, uninjured ones (Pl. 20). That these are not cases of stimulation of fungous growth by formaldehyde, with subsequent death of the seeds from the Rhizopus infection, can be easily shown by sterilizing the seeds with mercuric chlorid before germinating in sterile blotters. The injured seed lots appear just as evident as before, although in the absence of the fungus a higher percentage of germination usually is obtained. Thus, our experiments did not show any increase in susceptibility because of formaldehyde treatment but rather because of lowered viability of the seed. As explained earlier in this report, low viability, no matter how induced, always renders seeds susceptible to invasion by fungi.

That this relation between formaldehyde injury and fungous attack is not often illustrated by the development of Penicillium is due to the latter's peculiar sensitiveness to this chemical. On the other hand, if seeds are injured by copper sulphate, it is Penicillium, not Rhizopus,

which attacks them in the blotters. Rhizopus rarely attacks seeds which have been treated with copper sulphate, even if the seed coat is broken over the endosperm.

The susceptibility to Penicillium of seeds injured by copper sulphate is shown as strikingly in barley as in wheat. The seeds uninjured by the chemical escape attack unless, of course, the testa is broken over the endosperm.

Seed stored without drying after treatment with a 1 to 320 formaldehyde solution is much more resistant to the attack of molds than is untreated seed similarly stored. This is shown by the following experiment. Samples of barley and wheat were treated in the usual way by a 10-minute dip followed by a drain of 10 minutes. The excess moisture was removed and the seeds were dusted with Penicillium spores, as were also control lots treated in a like manner with water. All were sealed in small screw-top bottles. As was shown recently in another paper (*13*), this formaldehyde treatment does not injure the seeds so long as they are not allowed to dry with consequent formation of paraformaldehyde. After one month, samples of each lot were germinated, with the results shown in Table III.

TABLE III.—*Germination of wheat and barley soaked in formaldehyde solution and in water, followed by dusting with Penicillium spores*

Sample No.	Barley.		Wheat.	
	1 to 320 formaldehyde.	Water.	1 to 320 formaldehyde.	Water.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1	88	72	62	32
2	90	76	84	56

Copper sulphate has an even stronger inhibiting effect than formaldehyde on the development of molds on wheat in damp storage. In our experiments, however, Penicillium developed slowly in such seed, while it more rarely appeared in the seed stored similarly after formaldehyde treatment. Inhibition of Penicillium by the latter apparently gave opportunity for Aspergillus to develop. Aspergillus appears to develop most conspicuously when unfavorable factors to which Penicillium is more sensitive prevent the development of the latter. As a result, stored seed treated with copper sulphate has been destroyed largely by Penicillium and the formaldehyde-treated seed by Aspergillus, although both lots were stored under identical conditions.

The seed used in these experiments, one of which is summarized in Table IV, was machine-thrashed Little Club wheat in good condition, about 30 per cent of the grains having the seed coats broken over the embryo but with practically no endosperm injury. The varying degrees

of fungous attack, therefore, can be attributed solely to sensitiveness of the molds to the chemicals on the seed and are in no way complicated by treatment injury to the embryo. The formaldehyde-treated seed gave normal germination until destroyed by the molds; and the copper-sulphate-treated seed, while averaging only 84 per cent germination after treatment, was more resistant to molds than was the less injured, formaldehyde-treated seed.

TABLE IV.—*Relative development of molds on Little Club wheat stored two months under varying conditions of temperature and seed treatment*

Seed treatment.	Storage.	Relative heaviness of attack by molds.[a]	Penicillium.	Aspergillus.
Water	Refrigerator, 10° C.	+++	Present	Present.
Do	Laboratory, 20° C.	++++	....do	Do.
Do	Greenhouse, 15° to 35° C.	+++	....do	Do.
1 to 320 formaldehyde.	Refrigerator	++	Not present.	Do.
Do	Laboratory	++++	....do	Do.
Do	Greenhouse	+	....do	Do.
1 pound $CuSO_4$ to 4 gallons water.	Refrigerator	o	....do	Not present.
Do	Laboratory	+++	Present	Present.
Do	Greenhouse	o	Not present.	Not present.

[a] The symbols used indicate that the fungus is present (+) or lacking (o), the number of plus signs showing the relative severity of attack.

From these experiments it appears that seed treatments with copper sulphate and formaldehyde are a protection against saprophytic fungi, both when the seed is germinated at once and when it is stored damp. Formaldehyde is especially effective against Penicillium.

RELATION OF SEED-COAT INJURY TO INJURY FROM FUNGICIDES

The fact that all seed injury from the recommended copper-sulphate treatments for smut is due to broken seed coats seems to be unknown to most agronomists in this country. In only two bulletins (*12*, *25*) has the writer found mention of mechanical injury in discussions of the injurious effects of copper sulphate, or any attempt to correlate the various percentages of injury with the physical condition of the seed coat. Yet the principle of semipermeability of wheat and barley seed coats is not a new one. Brown (*3*) reported the discovery that the coverings of the seeds of *Hordeum vulgare* are semipermeable membranes, excluding salts and other substances from the seeds. He reported that there was no penetration of copper sulphate into seeds after soaking three days in a 5 per cent solution. In 1909 (*4*) he published the results of further experiments on the impenetrability of seed coats to salts. Fischer (*9*) soaked Sagittaria fruits five days in molecular copper sulphate without injury.

Reichard (*16*, *17*) did not find barley seed coats so perfectly semipermeable as those Brown described, and he attributes such variations to differences in the tannin content of the testa. He believes that solubility or nonsolubility of this tannin in the external solution determines the latter's penetrability. Differences in the tannin content of the active cells occur as the result of differences in the ripening process, he thinks, unripe seeds being more permeable because the tannin has not been fully deposited. It is interesting in this connection to note Falke's (*8*) conclusions as to the cause of the poor stand of wheat obtained from seed grown in an abnormally dry season. Not only was the wheat from untreated seed grown that year less vigorous, but when the seed was treated with a 0.5 per cent solution of copper sulphate it was extremely injured, while seed of the same varieties grown the preceding year showed much less effect of the poison. He concludes that the dryness of the season had affected the development of the testa with the result that it was more permeable to the poison. In all tests seeds with unbroken coats were selected for treatment.

In his studies on the semipermeability of the seed coats of wheat and barley, Schroeder (*18*, *19*) also found them to be impermeable to copper sulphate. Shull (*20*) thinks it reasonably certain that copper sulphate does not penetrate a sound testa. He attributes exceptions to defects in the seed coat too slight to be seen even on microscopic examination. Crocker and Davis (*6*) found that Alisma seeds would withstand a molecular copper-sulphate solution for a month.

These researches on the permeability of seed coats are comparatively recent, but it was known in 1872 that the injury to wheat resulting from treatment with copper sulphate was dependent on the physical condition of the seed coat. Nobbe (*15*) first recognized the fact that machine-thrashed seed was more injured by copper sulphate than was hand-thrashed seed, because damage done to the seed coats by the machine allowed the copper sulphate to penetrate to the embryo. He pointed out that the drier and more brittle the crop, the greater the thrashing injury. With the visibly injured kernels removed from the sample, he found germination to be as good as that of the hand-thrashed sample.

Kühn (*14*) reported no injury from copper sulphate when he used hand-thrashed wheat. Grassman (*10*) used machine-thrashed seed because it was what the farmers had to use, but he recognized the cause of the injury sustained by it and recommended that if in practice machine-thrashed seed had to be used, the strength of the copper-sulphate solution should be reduced. Falke (*7*) noted more treatment injury to machine-thrashed than to flail-thrashed grain. Von Tubeuf (*21*) found that machine-thrashed wheat would not stand treatment which was harmless to hand-thrashed grain. He, however, notes varying degrees of injury to hand-thrashed seed soaked 18 hours in a 2 per cent copper-

sulphate solution and suggests that the reason may be the presence of invisible imperfections in the testas. He discusses the difference in injury determined by germinating treated seed in various soils and in filter paper.

Volkart (*22*) gives the results of extensive experiments showing that mechanical injury to seed determines treatment injury. He relates the thrashing injury to the moisture content of the seed and shows further that the location of the break in the testa determines the degree of the injury done to the seed by a subsequent dip into copper sulphate. He points out that a break over the embryo exposes it to harmful, and in many instances fatal, action of the solution. If, however, the seed coat is ruptured over the endosperm only, the resultant injury is not serious. Burmester (*5*) said that the injuries received by the seed coat in thrashing made the seed very susceptible to copper-sulphate injury. He found that the higher the concentration of the solution used, the greater the percentage of injury. Woolman (*25*) says that most or all of the loss of germinative power of treated seed is due to thrashing injury. Wallden (*23*) located the mechanical injury to the seed coats by means of eosin, which does not penetrate a sound testa but enters every small fissure, staining the seed at that point. He determined that all breaks are relatively unimportant, except those occurring directly over the embryo. Seed with uninjured integuments could be exposed to copper-sulphate solutions of the highest concentrations without injury. Wallden also notes the greater susceptibility of broken grains to molds during storage.

In confirmation of all these results on the relation of thrashing injury of wheat to subsequent injury by copper sulphate, we may report here perfect germination of hand-thrashed White Australian, Sonora, Little Club, Early Baart, Marquis, Cedar, La Espiga, and Defiance wheat after treatment with a 1 to 4 solution (1 pound in 4 gallons) of copper sulphate. No sample of harvester-thrashed wheat examined with a hand lens in this laboratory has been found to be free from mechanical injury, the percentage of seed with the seed coats broken varying from 30 per cent to 100 per cent. The extent of the injury varies from an almost imperceptible crack to a large tear which leaves the whole end of the embryo exposed (Pl. 16). The percentage of germination of such samples after treatment varies directly with the percentage of seriously broken seed coats (Pl. 18, A). Shull (*20*) states that many seeds have defects invisible even under a microscope, and this may explain why apparently unbroken seeds occasionally are injured by copper sulphate.

Table V shows the immunity of the unbroken seed used in our experiments from injury by copper sulphate of any strength with ordinary exposures. Hand-thrashed Early Baart seed was exposed to a saturated solution of copper sulphate made by dissolving the copper sulphate in boiling water until a considerable amount crystallized upon cooling.

TABLE V.—*Germination of hand-thrashed wheat after immersion for various periods in saturated copper-sulphate solution*

	Duration of immersion in copper sulphate.					
	1½ hours.	6 hours.	8 hours.	10 hours.	16 hours.	Control, untreated.
Percentage of germination	100	100	92	82	25	100

As Wallden (*23*) and Volkart (*22*) report, only those cracks directly over the embryo permit injury from a 5-minute dip in a 1-pound to 4-gallon solution (Pl. 22, A). Our experiments also show that a similar dip in a saturated solution does not affect germination if the injury is over the endosperm. However, if longer exposures are made, the chemical eventually penetrates to the embryo with fatal results. After a soak of 1 hour in a 1-pound to 4-gallon solution, seeds with a scratch through the seed coat to the endosperm are slightly injured (usually the roots show some deformity or stunting), and after 6 hours the percentage of germination is low and growth of the seedling retarded. In a saturated solution, injury is extreme after 1 hour. Of course all seeds with the injury over the embryo are killed by these exposures in either strength. Seeds apparently unbroken are uninjured after 6 hours in a saturated solution, although there are occasional exceptions to this. The data from the experiments are presented in Table VI.

TABLE VI.—*Germination of wheat with broken and unbroken seed coats, as affected by copper-sulphate treatment*

Condition of seed and strength of solution.	Germination after exposure of—		
	5 minutes.	1 hour.	6 hours.
Seed coats unbroken:	*Per cent.*	*Per cent.*	*Per cent.*
1 to 4 solution	100	100	100
Saturated solution	100	100	[a] 100
Seed coats broken over endosperm:			
1 to 4 solution	100	92	68
Saturated solution		28	0
Seed coats broken over embryo:			
1 to 4 solution	58	0	0
Saturated solution	0	0	0

[a] The resistance of unbroken seeds to a 6-hour exposure in a saturated copper-sulphate solution varies with temperature and other conditions at present unknown.

The seedlings of all samples giving low germinations were very much deformed and stunted, the roots being especially injured (Pl. 23).

A lime dip usually increases the percentage of germination of injured seeds (Pl. 22, B). However, in badly broken seed, lime can not prevent copper-sulphate injury because the solution enters rapidly and injures

the embryo before it can be neutralized. Slightly damaged seed coats delay the entrance of the copper sulphate enough so that the lime saves the wheat.

There was more injury to the plumule when the seed was injured over the plumule end of the embryo than when it was injured over the radicle. The explanation is probably to be found in the fact that copper sulphate may not reach the inner end of the embryo at all, because of a bubble of air in the little pocket where the plumule lies which might protect it from being wetted by the liquid. When the seed was injured over the plumule, the copper sulphate had easier access to it and produced extreme deformity. When harvester-thrashed wheat is treated, the injury appears in the stunted roots of the seedling. More rarely is there a shortened or distorted plumule (Pl. 23).

An examination of several lots of harvester-thrashed seed shows great variation in the degree to which the seed coat over the embryo is injured. The question naturally arises as to whether the slight cracks which are barely visible allow a copper-sulphate solution to penetrate or whether the inner layer of the seed coat is a semipermeable membrane and keeps the copper sulphate out, even though the outer one is ruptured. A study of the seed coat over the embryo shows it to be composed of two layers which can be split apart and torn off separately. Bolley (*2*) gives a figure of a longitudinal section through a wheat kernel illustrating the difference in the structure of the coat over the embryo and the endosperm. To determine the semipermeability of the inner of these two layers, the outer was slit with a sharp needle so as to make a distinct tear, taking care not to injure the inner layer. Twenty-five such seeds were then dipped in a 1 to 4 copper-sulphate solution for 4 minutes and drained for 15 minutes. Another lot was broken in the same way but through both layers of the seed coat, and a control of uninjured seed was used (Pl. 22, A, 4). The germination percentages follow:

	Per cent.
Both layers of the seed coat over the embryo broken	30
Only the outer layer of the seed coat broken	90
Seed coat uninjured	100

It appears from this experiment that the inner layer is impermeable to copper sulphate and that little or no injury results to the seed in treating unless both layers are broken. The slightly lowered germination of the less-injured seeds no doubt is due to accidental injury to the inner layer. Thus is explained the fact that the percentage of mechanically injured seed in a harvester-thrashed lot is slightly higher than the percentage of such seed killed by treatment. The percentage of badly injured seed counted corresponds more nearly to the amount of injury to be expected when the sample is treated with a 1 to 4 solution.

It is interesting to compare the percentage of injured seed coats found in some of the samples of harvester-thrashed seed sent to the laboratory

with the germination obtained after treating them with 1 to 4 copper-sulphate solution (Table VII, Pl. 21, A).

TABLE VII.—*Data showing relation of broken seed coats to injury from a 5-minute dip in 1 pound to 4 gallons copper-sulphate solution, as indicated by germination*

Nature of sample.	Slightly broken seed coats.	Badly broken seed coats.	Germination after treatment.
	Per cent.	*Per cent.*	*Per cent.*
Little Club, harvester-thrashed	10	30	84
Do	12	88	48
Little Club, hand-thrashed	0	0	100
Early Baart, harvester-thrashed and scoured for milling	1	99	20
Early Baart, hand-thrashed	0	0	100

It is possible, therefore, by examining the wheat with a hand lens, to predict very closely the loss that will be caused by copper-sulphate treatment. If the seed is known to be infested with smut spores, as shown by the darkened brush or the presence of smut balls, it may become a problem as to which will do the greater damage, the copper sulphate or the smut. A way out of the difficulty may be found in the use of 1 to 320 formaldehyde solution (1 pint commercial formaldehyde in 40 gallons). A dip of 10 minutes in this solution, or in a 1 to 160 solution, followed by a 10-minute drain, does not injure the embryo, no matter how extreme the mechanical injury to the seed coat. A 1 to 80 solution, however, causes extreme injury when the seed coat is broken over the embryo, and only when it is so broken, but this strength never is recommended. It should be noted in explanation of these statements of the inability of the 1 to 320 and 1 to 160 strengths to injure even broken wheat that the so-called formaldehyde injury occasionally reported after the use of these strengths is caused by the formation of paraformaldehyde during the drying of the seed. If the seed is not dried but is sown moist in damp soil or kept in damp storage, no injury results from the treatment. Paraformaldehyde is very unstable and is constantly breaking down into formaldehyde gas. Thus the seed, unless well spread, is surrounded by this toxic vapor which penetrates the seed coat, probably by again going into solution in the presence of any moisture in the seed covering.

Seed-coat condition is important, therefore, in connection with this formaldehyde injury after the drying of treated seeds. The data presented in Table VIII show that, although a perfect seed coat delays injury from paraformaldehyde, it does not prevent it. The paraformaldehyde was obtained by evaporating some commercial formaldehyde and powdering the white residue in a mortar. The seeds were then placed in Syracuse watch crystals and covered with the perfectly dry powder, which was packed around them.

TABLE VIII.—*Relation of seed-coat condition to paraformaldehyde injury, as indicated by germination*

Length of exposure to paraformaldehyde.	Percentage of germination.		
	Little Club.		Early Baart, hand-thrashed.
	Hand-thrashed.	Harvester-thrashed.	
1 day	100	50	100
2 days	90	50	80
6 days	70	0	30
14 days	0	0	0

In conclusion, mechanical injury to the seed in thrashing governs seed injury to wheat resulting from treatment with copper sulphate and from saprophytic fungi, in storage and in the soil. It has been shown (1) that perfect seed coats are an absolute protection in ordinary exposures to copper sulphate of any strength, (2) that they are partial protection against injury which results when formaldehyde-treated seeds are dried, and (3) that they afford marked protection against saprophytic fungi in storage and in the soil. Better thrashing methods are the obvious remedy for the losses caused by seed treatment for smut and by molding of damp grain in storage or in cold, damp soil.

SEMIPERMEABILITY OF BARLEY SEED COATS IN COPPER-SULPHATE SOLUTIONS

It is often stated in agricultural bulletins, and farmers commonly believe, that barley is more sensitive to fungicides, notably copper sulphate, than is wheat. But if, as Brown (*3*, *4*) found, the seed coats are impermeable to copper sulphate, this should not be true. Experiments were undertaken, therefore, to determine whether there is any basis for this prevailing belief in the greater susceptibility of barley.

The first variety experimented upon was Turkestan barley, of which there happened to be a supply of unthrashed seed in good condition in the laboratory. The hand-thrashed seed was soaked in a saturated solution of copper sulphate for 6, 8, and 10 hour intervals, as had been done with wheat (see Table V). To our great surprise, all were killed, not a single seed showing any sign of germination, although in later experiments occasional exceptions to this were found (Table IX). It had been noticed that barley, unlike wheat, had a more or less ragged hole on each kernel where it was broken from the rachis. It appeared that when barley was thrashed, even by hand, every seed coat was weakened or injured at this point. It was assumed from this fact that the kernels left attached to a piece of the rachis would be as impermeable to copper sulphate as is wheat. Experiments proved this sup-

position correct. Heads were broken into fragments so that from one to three kernels were attached to each piece of the rachis. These were treated with saturated copper sulphate for 6 hours and for 10 hours. The resulting germination was 90 and 80 per cent, respectively. (The control germinated only 85 per cent.) None of the seedlings showed any abnormality. The kernels stripped from the rachis and treated in the same way were all killed (Pl. 21, B). The experiment shows the semipermeability of unbroken Turkestan barley kernels in saturated copper sulphate and the destruction of this semipermeability by mechanical injury at the hilum caused by breaking the kernel from the rachis.

Even more marked results were obtained upon treating some well-thrashed Coast barley with saturated copper-sulphate solution, the kernels being similar in structure to those of the Turkestan variety. Only 28 per cent germination was obtained after an exposure of one hour. Exposures of five minutes were found not to be injurious. Coast barley is the same subvariety (*Hordeum vulgare* var. *coerulescens*) (*11*) as the one used by Brown (*4*) and which he found to be impermeable to copper sulphate after three days in a 5 per cent solution. Similar results were obtained with Turkestan barley and are shown in Table IX, together with the germination percentages of Early Baart wheat obtained after the same treatments. The comparison brings out the greater susceptibility of this hand-thrashed barley to copper-sulphate injury, compared with hand-thrashed wheat.

TABLE IX.—*Comparative germination of hard-thrashed barley and wheat after treatment with saturated copper-sulphate solutions*

Length of exposure.	Turkestan barley.		Early Baart wheat.	
	Germination.	Height of plumule.	Germination.	Height of plumule.
	Per cent.	*Cm.*	*Per cent.*	*Cm.*
5 minutes	100	12	100	8
1 hour	100	12	100	8
6 hours	60	7	96	8
24 hours	0	.....	52	2
Control, untreated	100	12	96	8

If it is the nature of the abscission of the barley kernel from the rachis which determines its resistance to copper-sulphate injury, it follows that those varieties which shatter easily in the field and in which a smoother, more natural break occurs between kernel and rachis should show more perfect semipermeability. The Nepal variety (White Hull-less) seemed to be of this type. On treating, however, our samples showed even greater injury than that sustained by the nonshattering Coast barley. It is to be regretted that lack of sufficient hand-thrashed material of these

and other varieties has prevented further experiments on the relation of morphology of the heads to the susceptibility of the seeds to fungicides.

These experiments on semipermeability of barley seed coverings were made with more concentrated solutions than are used in farm practice. It seemed desirable to determine the effect of the recommended strength, 1 pound to 4 gallons, on different varieties, after the usual immersion of about five minutes. The varieties found to germinate normally after this treatment were Tennessee Winter, Mariout, Beldi, Turkestan, and Coast. Mariout, Beldi, and Coast showed slight retardation and root injury, but this would not be serious in soil germinations. However, the Nepal (White Hull-less) variety was more seriously injured, with retarded plumules, stunted roots, and a germination of only 80 per cent against a control germinating 100 per cent.

LIMITATIONS AND VARIATIONS IN SEMIPERMEABILITY OF SEED COATS OF WHEAT

Wheat seeds are injured and killed by prolonged exposure to copper-sulphate solutions in spite of the fact that their testas are reported without exception to be semipermeable membranes. Brown (3) says that grains of *Hordeum vulgare* were not injured by a 3-day exposure to a 5 per cent solution of copper sulphate, but we have found no sample of either wheat or barley which would stand an exposure of even 1 day in a 1-pound to 4-gallon solution without injury, while death resulted in many cases. Reference to Table V will show the endurance of one of our best wheat samples in a saturated solution. It was found in this as well as in many repetitions that while hand-thrashed seed may be uninjured after 6 hours in this solution, injury is decided after 8 hours, and but a small percentage of seedlings, all retarded or deformed, are obtained from seed exposed for 16 or 24 hours. In many cases, extreme injury was produced after an exposure of only 6 hours. The data in Table X illustrate the variations and the limitation of the phenomenon of semipermeability in wheat.

To the data in Table X should be added the fact that no germination was obtained in thrashed Turkestan (see Table IX for exception), Tennessee Winter, Beldi, Nepal, and Mariout barleys after immersions of six hours in saturated copper-sulphate solutions, although barley kernels of these types always are reported as inclosed in semipermeable membranes. We shall exclude barley, however, from this discussion, as it has been shown that the nature of the abscission of the kernel may have something to do with its permeability.

TABLE X.—*Germination of hand-thrashed seed wheat treated for various periods in copper-sulphate solutions*

Experiment No.	Variety.	Strength of solution.	Length of treatments and germination obtained.	Germination of control.
				Per cent.
1	Little Club	Saturated	1½ hours, 100 per cent; 16 hours, 40 per cent.	100
2	do	do	2 hours, 93 per cent; 6 hours, 86 per cent; 24 hours, 0.	100
3	do	do	16 hours, 5 per cent	100
4	Early Baart	do	6 hours, 100 per cent; 8 hours, 92 per cent; 10 hours, 82 per cent.	100
5	do	do	6 hours, 80 per cent; 10 hours, 70 per cent.	100
6	do	do	6 hours, 96 per cent; 24 hours, 52 per cent.	96
7	do	do	2 hours, 95 per cent; 4 hours, 85 per cent; 8 hours, 80 per cent; 24 hours, 35 per cent.	.....
8	do	do	½ hour, 75 per cent; 8 hours, 35 per cent; 24 hours, 10 per cent.	.....
9	do	do	6 hours, 8 per cent	84
10	do	1 pound to 4 gallons.	6 hours, 96 per cent; 24 hours, 72 per cent.	96
11	do	do	8 hours, 90 per cent	100
12	La Espiga	Saturated	6 hours, 5 per cent	94
13	Cedar	do	6 hours, 50 per cent	98

The data here reported on wheat indicate either (1) that the seed coat is not perfectly semipermeable in copper-sulphate solutions but allows a slow diffusion of the salt into the seed, or (2) that the semipermeability of the seed coat is destroyed after a limited exposure, either by chemical injury from the poison, by stretching due to the absorption of water, or by some other means. Reichard (*16*) and Bokorny (*1*) found barley testas not so perfectly semipermeable as is reported by Brown (*3, 4*). Von Tubeuf (*21*) and Falke (7) both report penetration of copper sulphate through apparently sound testas of wheat. Shull (*20*) says that ultramicroscopic defects in the seed coats of Xanthium make quantitative data approximations only.

> Since 60–80 per cent of the seeds show no penetration after prolonged soaking in $CuSo_4$ and since they retain their vitality perfectly . . . it seems reasonably certain that $CuSo_4$ does not penetrate a sound testa.

He seems to regard membranes as semipermeable when they exclude substances for days, weeks, and months, and as permeable when the exclusion is merely a matter of hours. On this basis the testa of wheat is not semipermeable, or at least its semipermeability has very narrow time limits.

The amount of injury to samples of hand-thrashed Early Baart wheat caused by long exposures to copper-sulphate solutions varies directly as the concentration of the solution—that is, the stronger the copper sulphate the greater the percentage of injured seeds. Individual seeds vary in their resistance to penetration, some succumbing before others, so there is a gradual increase in germination with increased dilution of the solution. The data in Table XI illustrate this.

TABLE XI.—*Relation of strength of copper-sulphate solution to seed injury after an 8-hour exposure as indicated by germination and seedling growth of Early Baart wheat*

Strength of solution.	Germination.	Height of plumule.
	Per cent.	*Cm.*
Saturated	30	5.5
1 pound to 1 gallon	55	6.5
1 pound to 4 gallons	90	10.0
1 pound to 10 gallons	85	10.0
1 pound to 80 gallons	100	10.0
Control, untreated	100	10.0

The great variations in the length of time seeds from the same sample of wheat could be soaked in copper-sulphate solutions without injury have been very perplexing throughout our experiments. The preceding data bring out the fact that although, as a rule, penetration of the poison is first noticeable after about 6 hours and not decided until after 8 hours, occasionally almost no germination was obtained after 6 hours, and injury appeared even sooner. In some experiments, no germination was obtained after 24 hours' exposure, while in others there was only about 50 per cent. It was thought possible that these variations might be due, at least in part, to differences in temperature. Consequently, some experiments were planned to show the relation of temperature of the solution to the length of time hand-thrashed Early Baart wheat could remain in a saturated copper-sulphate solution without injury. Table XII gives the results of one of several experiments.

TABLE XII.—*The relation between temperature and the semipermeability of the seed coats of Early Baart wheat in a saturated copper-sulphate solution, as indicated by germination*

Length of exposure.	Germination when stored at —				
	9° C., in refrigerator.	13.5° C., outside window ledge.	17° C., in laboratory.	35° C., in incubator.	55° C., in incubator.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
2 hours	95	85	95	90	85
4 hours	90	90	85	80	65
8 hours	95	75	80	75	55
24 hours	70	55	35	40	30

The data in Table XII indicate that wheat will withstand the penetration of a saturated copper-sulphate solution for a longer time at the low temperature of the refrigerator than at the higher ones of the laboratories and incubators. If the increased permeability occurs as the result of chemical changes, we might expect a very exact relationship between temperature and the resistance of the seed to the penetration of copper sulphate. The preceding experiment and others indicate some such correlation.

SUMMARY

(1) An unbroken seed coat ordinarily affords absolute protection against attack of living seeds by Penicillium or Rhizopus in damp storage, in the soil, or in blotter germinations. Infection of such seeds has been obtained, however, by retarding germination of the seed by means of low temperatures.

(2) The location of a break in the seed coat determines the ability of saprophytic fungi to invade seeds, either in the soil, in storage, or in blotter germinations. If the injury is over the endosperm, 100 per cent fatal infection results when the spores of Penicillium or Rhizopus are present; but if it is over the embryo, the seeds remain practically immune.

(3) The vitality of seeds is a factor in determining the ability of Penicillium and Rhizopus to attack them. Death or injury resulting from seed treatment, or other cause, renders previously immune seeds immediately susceptible. Even perfect seed coats no longer are a protection.

(4) No visible infection with either Penicillium or Rhizopus occurred where the temperature remained as low as 10°C. throughout the experiment.

(5) Penicillium requires an atmospheric humidity of at least 80 per cent for its development on stored wheat. Aspergillus will grow on wheat at a humidity of 70 per cent.

(6) These molds develop more slowly on stored wheat and barley which has been treated with copper sulphate or formaldehyde than on equally moist untreated wheat. Penicillium is especially sensitive to formaldehyde.

(7) Although a break in the testa over the endosperm of wheat does not result in any injury to the germ upon short exposures to copper sulphate, injury becomes apparent after exposures of an hour, showing that the poison eventually is absorbed through the endosperm and scutellum. A similar break over the embryo results in its death after exposures of only three to five minutes.

(8) When seed coats are badly injured, liming does not prevent extreme injury, because the copper sulphate enters such seeds quickly. When the seeds are only slightly injured, a lime dip is effective in neutralizing the solution before injury occurs.

(9) The outer layer of the seed coat may be broken over the embryo without injury resulting from treatment with copper sulphate, the inner layer being impermeable to it.

(10) Stunted roots, rather than injured plumules, are characteristic of copper-sulphate injury, because machine thrashing usually breaks the seed coat directly over the radicle.

(11) The damage that will be done to seed wheat by the copper-sulphate treatment for smut and by saprophytic fungi can be predicted by examination of the physical condition of the seed. All these troubles can be reduced by greater care in thrashing the seed wheat so that the seed coats are not so badly broken.

(12) Perfect seed coats are also an absolute protection against short exposures to strong formaldehyde solutions and are partial protection against post-treatment injury after disinfection with formaldehyde.

(13) The seed coats of Turkestan barley and varieties of the same structural type are broken at the hilum in thrashing, either by machine or by hand. Death results from exposing such kernels to strong copper-sulphate solutions for even short periods or to weaker solutions for longer periods. Barley kernels left attached to pieces of the rachis were not injured by these treatments.

(14) Injury to wheat seed always occurred after exposures to saturated copper-sulphate solutions for periods longer than six hours and sometimes in less time. This fact raises a question as to the perfect semipermeability of the testas of wheat to this poison.

(15) Temperature of the solution was found to be a factor in the resistance of wheat to injury from long immersions in saturated copper-sulphate solutions, the germination being poorer as the temperature increased.

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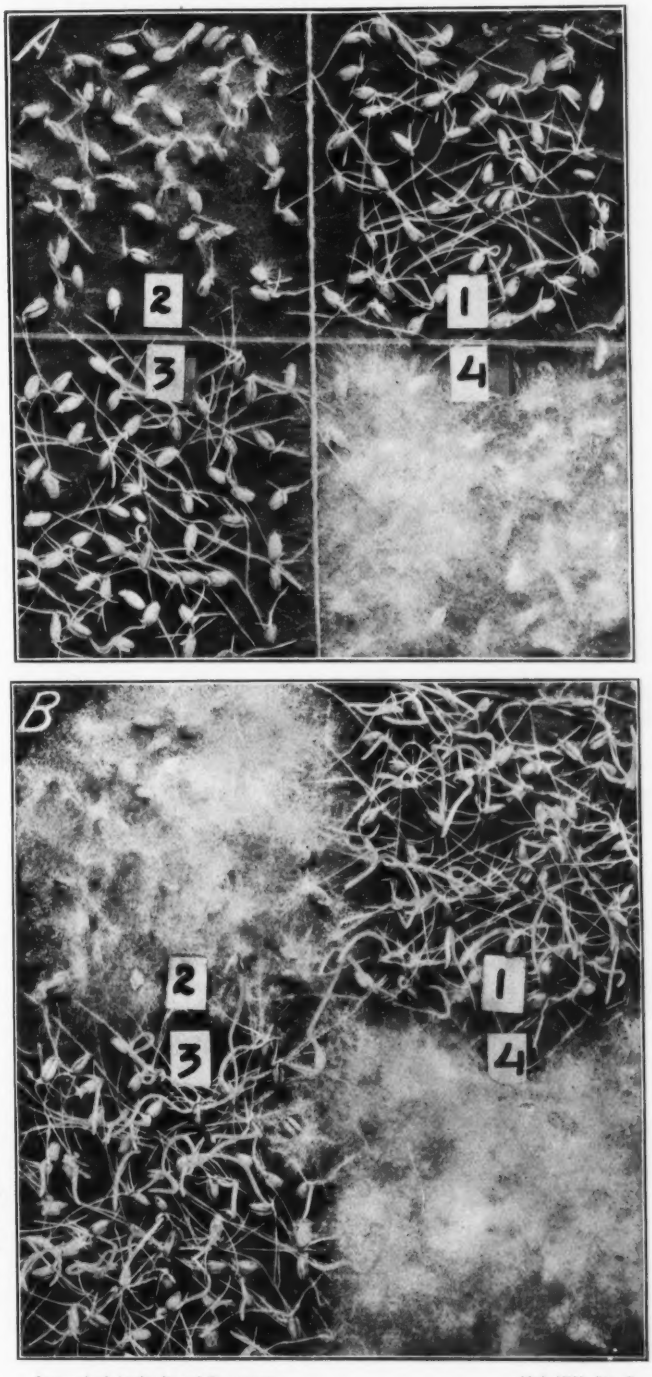
PLATE 13

A.—Relation of seed-coat injury to attack by Rhizopus on seed germinating on an infected blotter: 1, hand-thrashed seed, seed coats uninjured; 2, hand-thrashed seed, seed coats broken over endosperm; 3, hand-thrashed seed, seed coats broken over embryo; 4, harvester-thrashed seed scoured for milling.

B.—Same as A but 2 days later, showing that the badly infected seeds never produce plants.


A
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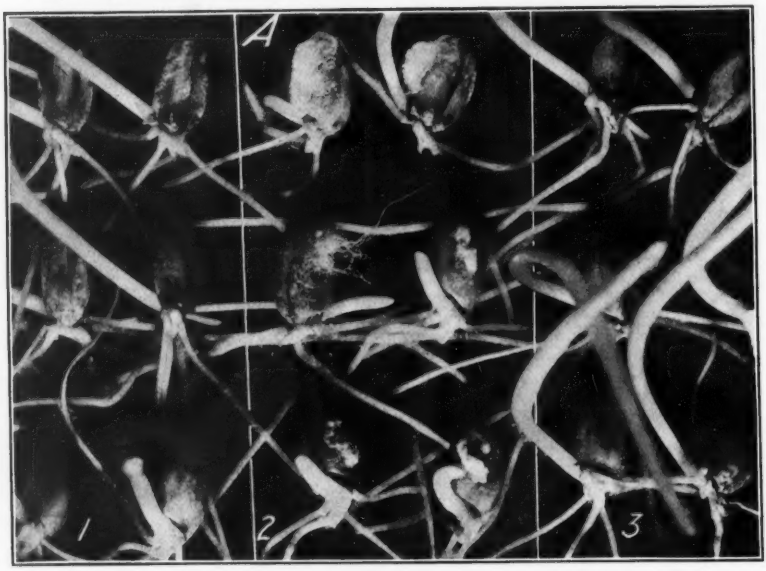
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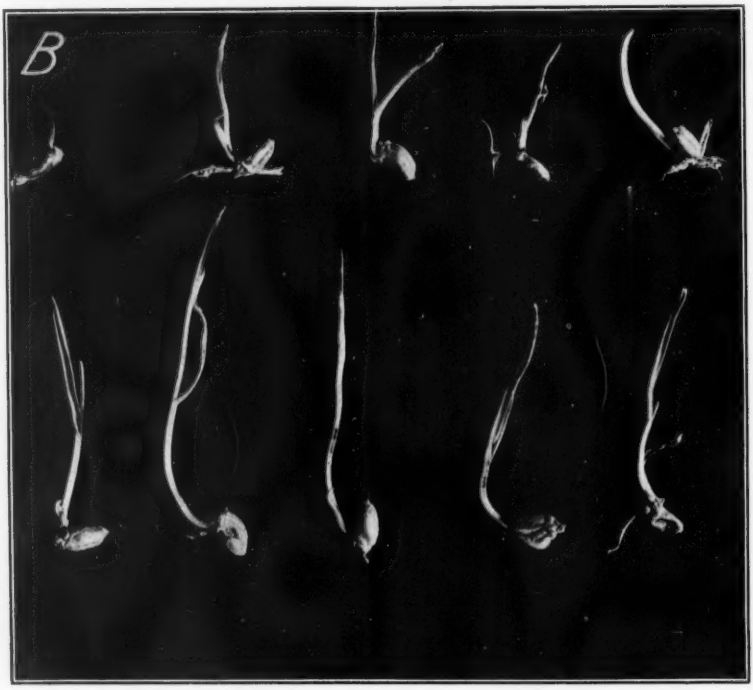
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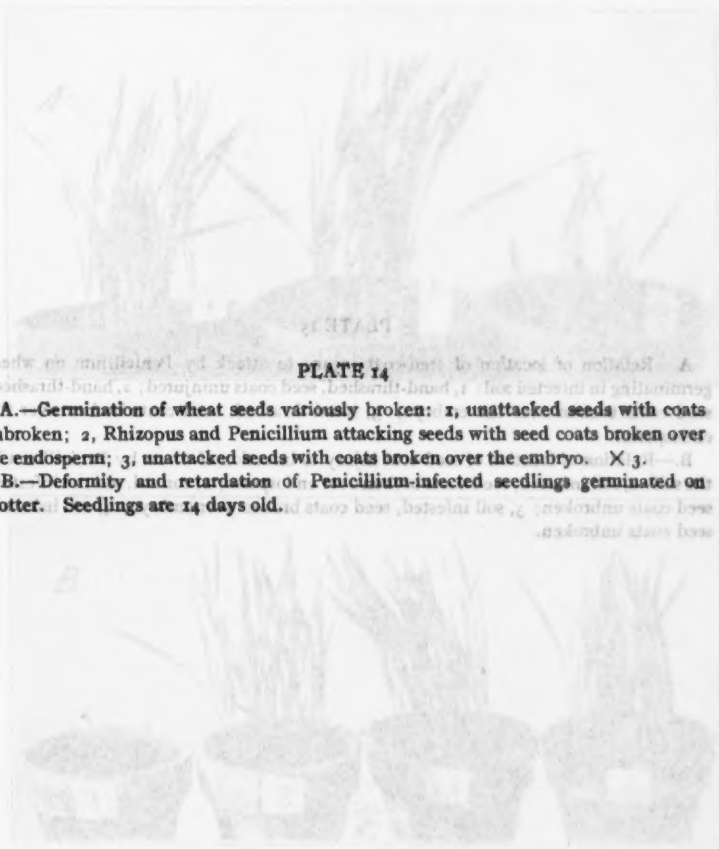


PLATE 14

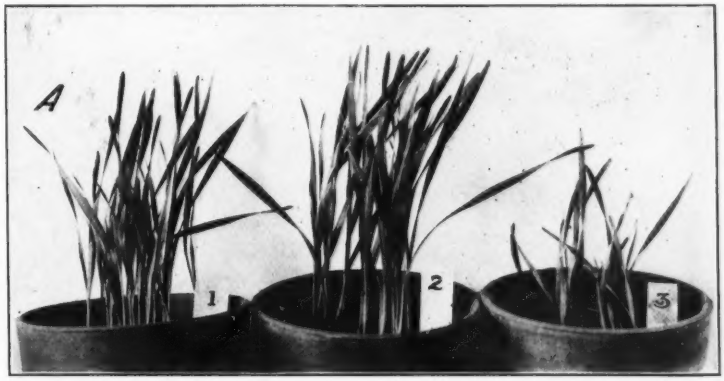
A.—Germination of wheat seeds variously broken: 1, unattacked seeds with coats unbroken; 2, Rhizopus and Penicillium attacking seeds with seed coats broken over the endosperm; 3, unattacked seeds with coats broken over the embryo. × 3.

B.—Deformity and retardation of Penicillium-infected seedlings germinated on blotter. Seedlings are 14 days old.

PLATE 15

A.—Relation of location of seed-coat injury to attack by Penicillium on wheat germinating in infected soil: 1, hand-thrashed, seed coats uninjured; 2, hand-thrashed, seed coats broken over the embryo; 3, hand-thrashed, seed coats broken over the endosperm.

B.—Relation of location of seed-coat injury to infection of wheat by Penicillium in the soil: 1, soil infested, seed coats broken over endosperm; 2, control, soil not infested, seed coats unbroken; 3, soil infested, seed coats broken over embryo; 4, soil infested, seed coats unbroken.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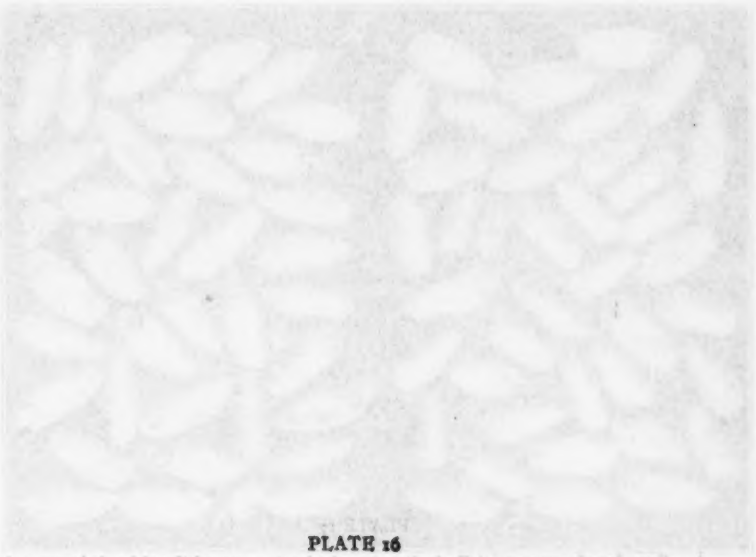


PLATE 16

Thrashing injury to Little Club wheat:
A.—Seed coats uninjured.
B.—Seed coats broken.
× 12.

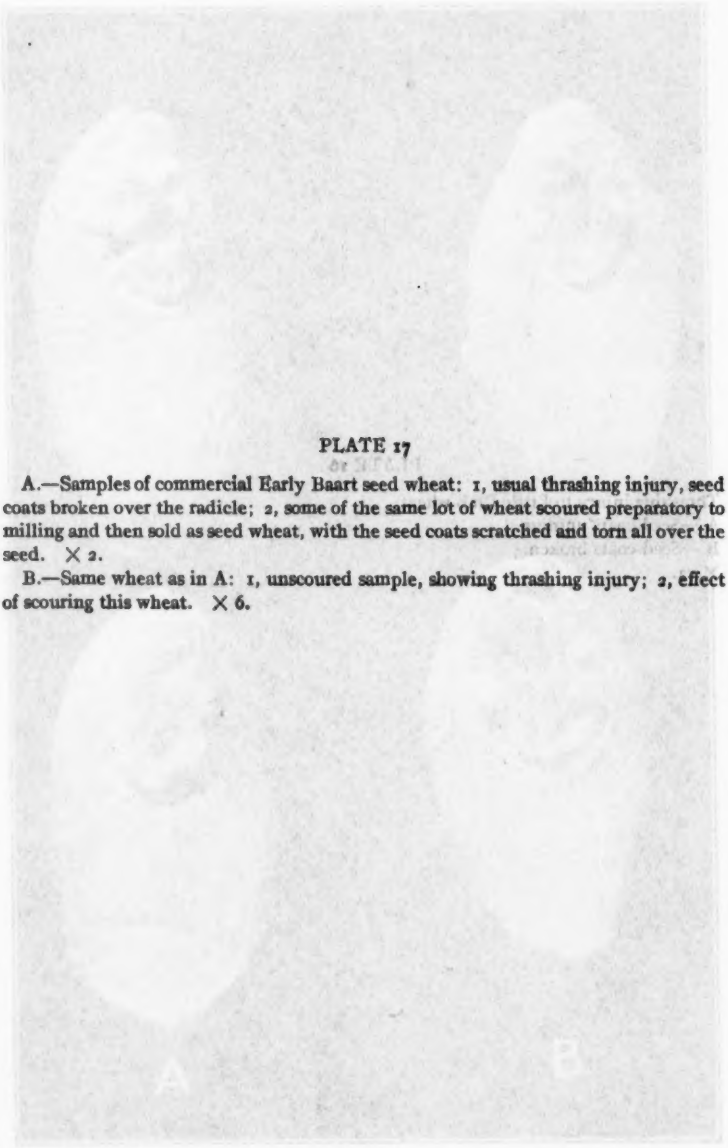
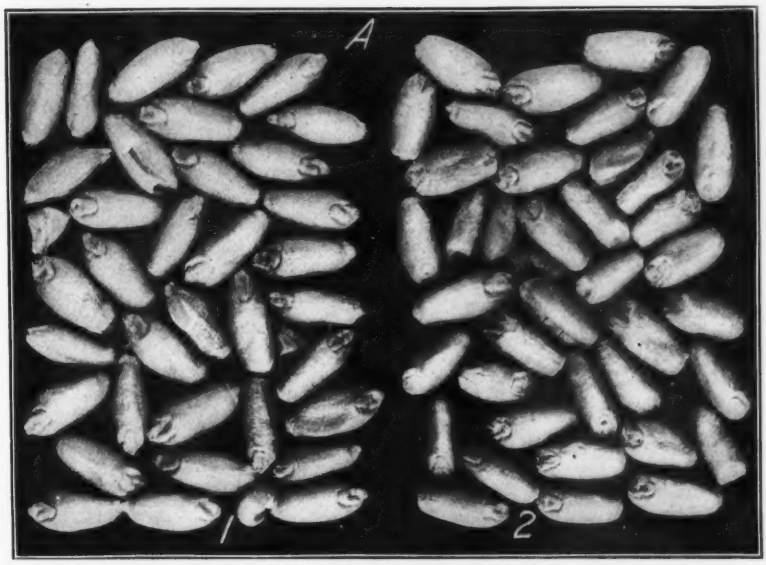


PLATE 17

A.—Samples of commercial Early Baart seed wheat: 1, usual thrashing injury, seed coats broken over the radicle; 2, some of the same lot of wheat scoured preparatory to milling and then sold as seed wheat, with the seed coats scratched and torn all over the seed. × 2.

B.—Same wheat as in A: 1, unscoured sample, showing thrashing injury; 2, effect of scouring this wheat. × 6.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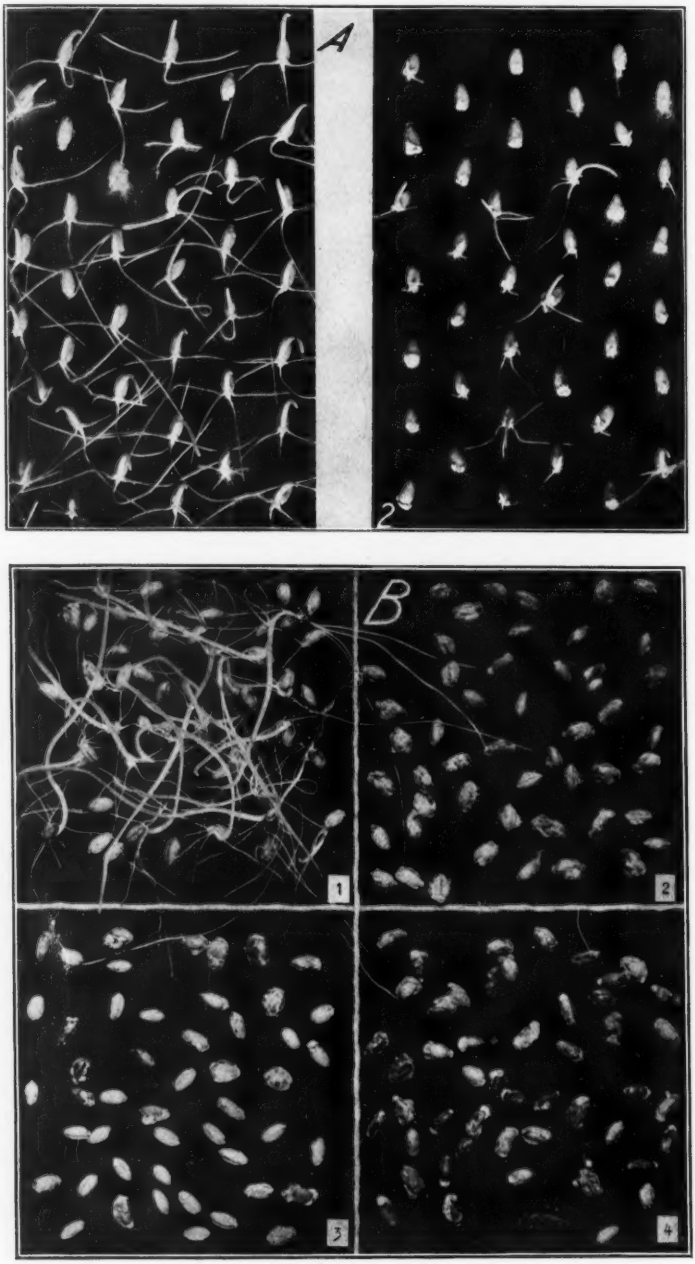
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PLATE 18

A.—Germination of the untreated wheat illustrated in Plate 17: 1, harvester-thrashed seed, germination 90 per cent; 2, same seed after scouring, germination 46 per cent, and showing growth of Penicillium and Rhizopus which gained entrance through the scratched and broken seed coats.

B.—Relation of seed-coat injury to destruction of damp, stored, untreated wheat by molds, the seeds having been sprinkled with spores and stored for 10 weeks in bottles in a damp chamber: 1, seed coats unbroken; 2, seed coats broken over endosperm; 3, seed coats unbroken but seeds previously killed by boiling; 4, seed coats broken over embryo.

PLATE 19

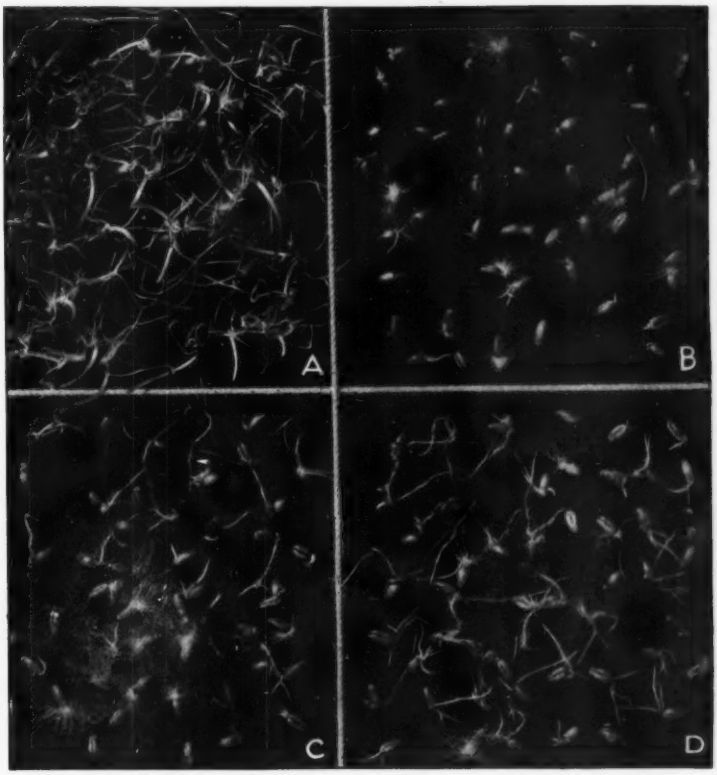
Increased susceptibility to Rhizopus of seeds injured by formaldehyde when dried after treatment with a 1 to 320 solution and stored for 18 days.

A.—Seed uninjured by treatment, having been stored damp.

B.—Seed killed, having been sealed after drying 7 hours following treatment.

C.—Seed nearly killed, having been sealed after drying 24 hours following treatment.

D.—Seed badly injured, having been sealed after drying 3 days following treatment.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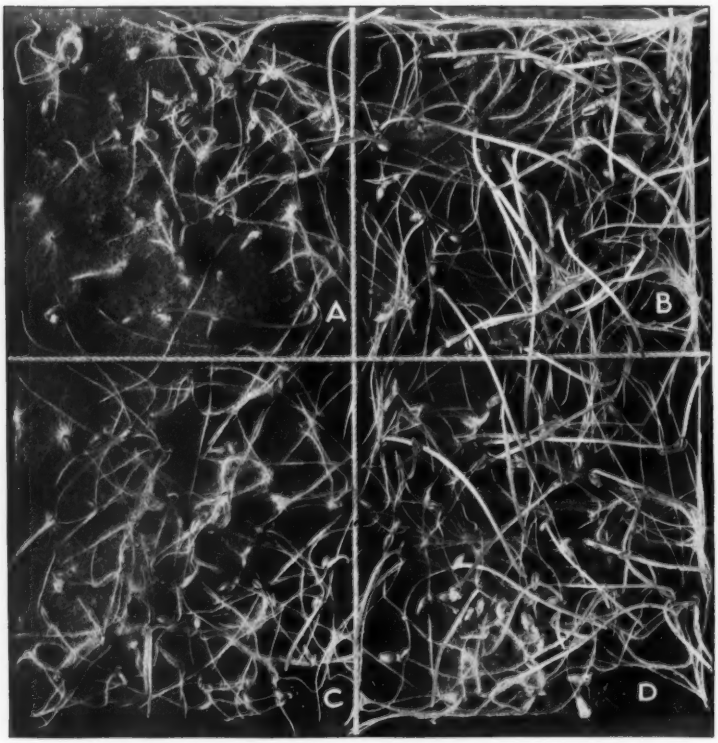
A
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PLATE 20

Increased susceptibility to Rhizopus of seed injured by dry storage after formaldehyde treatment. After treatment some of the seeds were washed in water, some left unwashed, and all were dried for one month.

A.—Treated with 1 to 20 formaldehyde, dried unwashed; germination 32 per cent.

B.—Treated with 1 to 20 formaldehyde, washed before drying; germination 76 per cent.

C.—Treated with 1 to 40 formaldehyde, dried unwashed; germination 52 per cent.

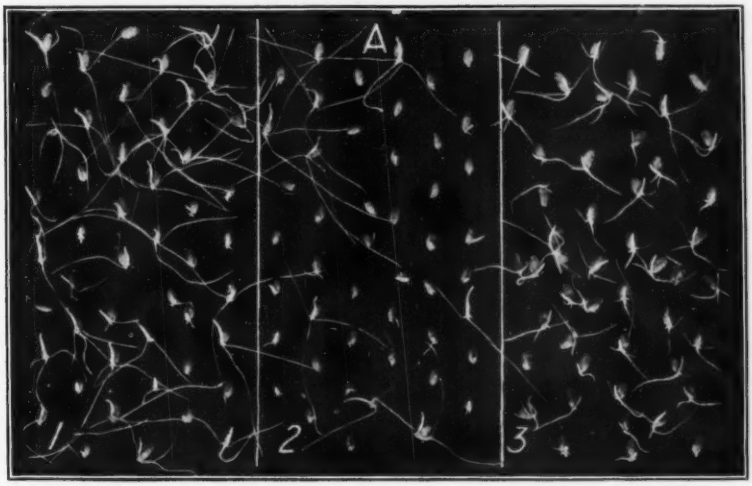
D.—Treated with 1 to 40 formaldehyde, washed before drying; germination 74 per cent.

Control germinated 74 per cent.

PLATE 21

A.—Relation between percentage of seed-coat injury and degree of injury from a 5-minute exposure to a 1-pound to 4-gallon solution of copper sulphate: 1, harvester-thrashed seed, with 30 per cent of seed coats broken, germination 86 per cent; 2, harvester-thrashed seed, with 95 per cent of seed coats broken, germination 42 per cent; 3, hand-thrashed seed, seed coats unbroken, germination 98 per cent.

B.—Susceptibility of wheat and barley to long exposure to a saturated copper-sulphate solution: 1, Early Baart wheat exposed 6 hours, germination 80 per cent, 2, Early Baart wheat exposed 10 hours, germination 80 per cent; 3, Early Baart wheat, control, untreated, germination 95 per cent; 4, Turkestan barley, control, untreated, germination 100 per cent; 5, Turkestan barley, kernels stripped from rachis, exposed 6 hours, no germination; 6, Turkestan barley, kernels left attached to piece of rachis during treatment, exposed 6 hours, germination 90 per cent; 7, Turkestan barley, kernels stripped from rachis, exposed 10 hours, no germination; 8, Turkestan barley, kernels left attached to piece of rachis during treatment, exposed 10 hours, germination 90 per cent.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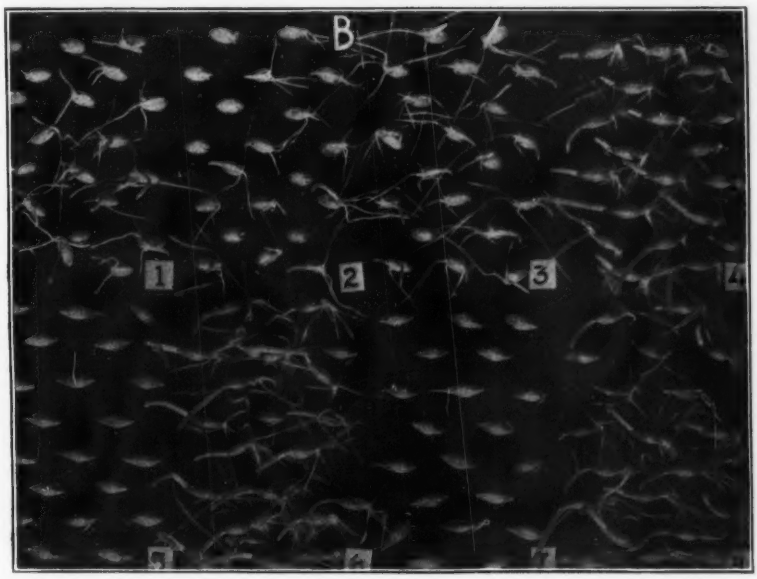
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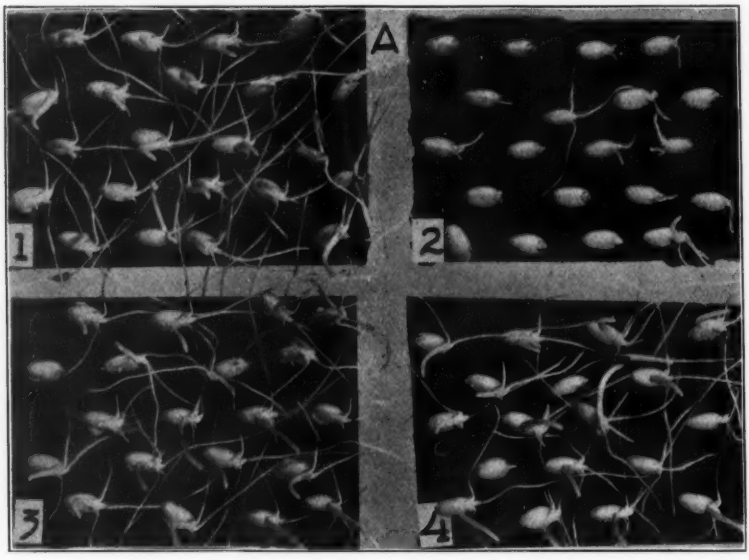
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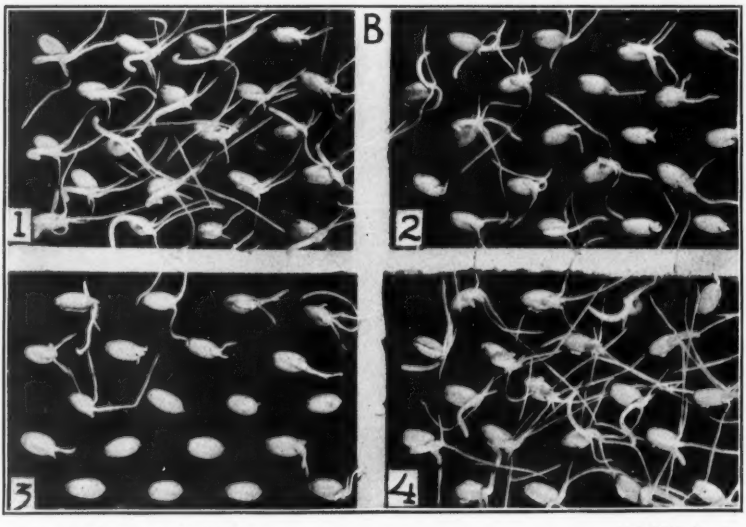
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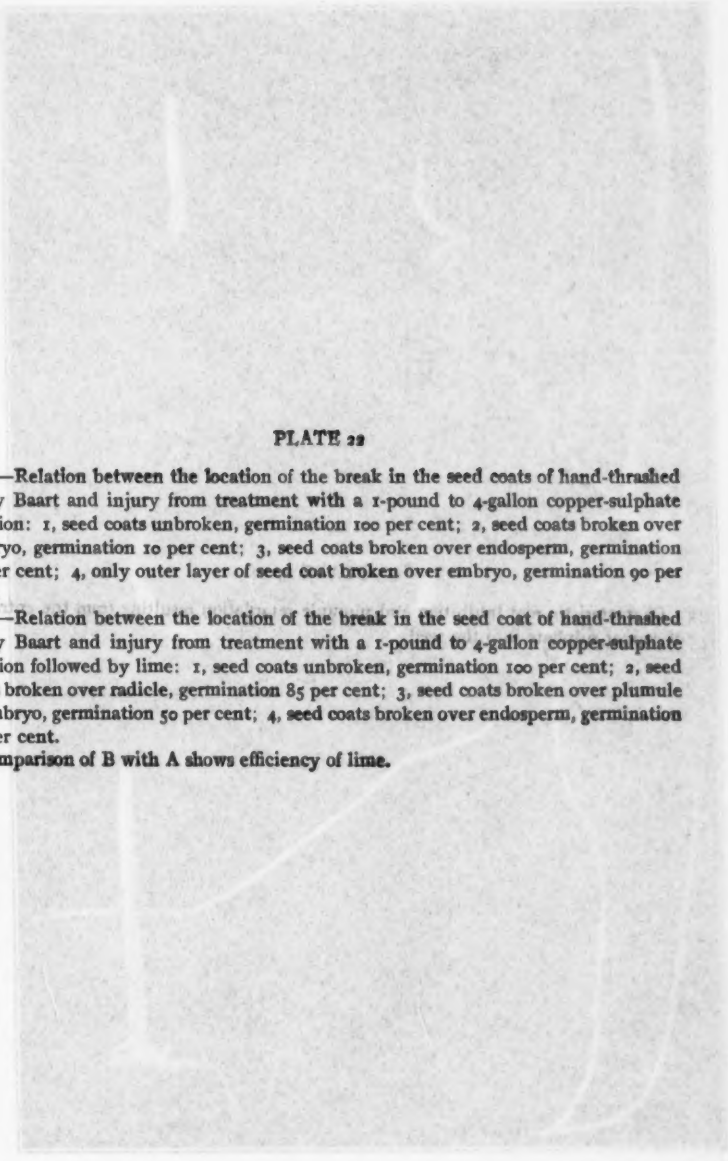

PLATE 22

A.—Relation between the location of the break in the seed coats of hand-thrashed Early Baart and injury from treatment with a 1-pound to 4-gallon copper-sulphate solution: 1, seed coats unbroken, germination 100 per cent; 2, seed coats broken over embryo, germination 10 per cent; 3, seed coats broken over endosperm, germination 95 per cent; 4, only outer layer of seed coat broken over embryo, germination 90 per cent.

B.—Relation between the location of the break in the seed coat of hand-thrashed Early Baart and injury from treatment with a 1-pound to 4-gallon copper-sulphate solution followed by lime: 1, seed coats unbroken, germination 100 per cent; 2, seed coats broken over radicle, germination 85 per cent; 3, seed coats broken over plumule of embryo, germination 50 per cent; 4, seed coats broken over endosperm, germination 95 per cent.

Comparison of B with A shows efficiency of lime.

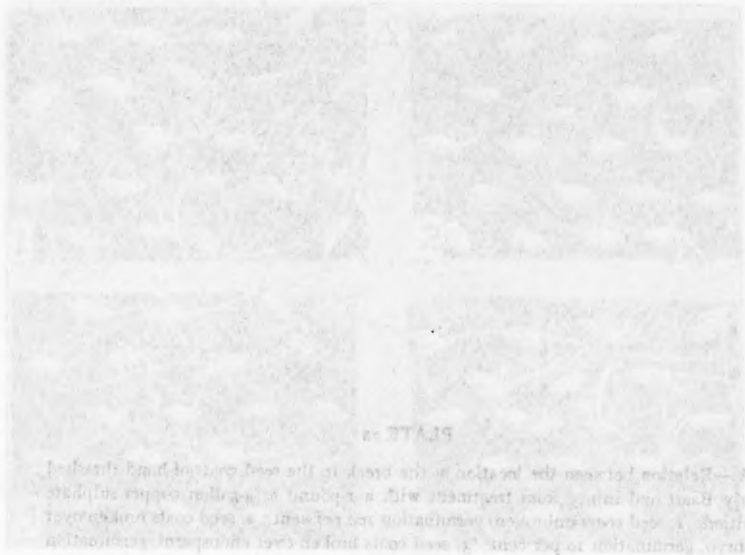

PLATE 23

Characteristic root inhibition and plumule retardation resulting from the entrance of copper sulphate into the seed.

BACTERIAL SPOT OF TOMATO[1]

By MAX W. GARDNER, *Associate in Botany*, and JAMES B. KENDRICK, *Assistant in Botany, Purdue University Agricultural Experiment Station*

INTRODUCTION

During recent years the tomato crop in the north central States has been affected with a spot disease of the fruit which was recognized by pathologists as distinctly different from any of the well-known tomato fruit spots. W. A. Huelsen, of the Purdue Agricultural Experiment Station, noted the prevalence of this disease in the Indiana canning tomato crop of 1918. In the 1919 crop the disease again became prevalent in Indiana, and the present study was begun in the fall of 1919 and continued throughout the winter and spring. The present work deals mainly with the symptoms of the disease, the isolation, pathogenicity, cultural characters, overwintering, and dissemination of the causal bacteria, and their relation to the host tissue. Certain phases relative to the mode of fruit infection under field conditions are to receive further study.

The relation of this tomato disease to the bacterial spot of pepper which occurs in Florida has received only preliminary study. Apparently the causa. organisms are identical.

THE DISEASE

NAME

This disease as it occurs on the fruit has been called "canker" by Coons and Nelson (*4*, *p. 48*)[2] and "scab," "fruit scab," and "black scab" by McCubbin (*9*, *p.* 15). It seems advisable, however, to adopt the name "bacterial spot" for the disease, since the term canker has been used for another tomato disease, and since the term scab is hardly applicable to the foliage lesions. The name bacterial spot has also been used for the related disease of peppers (*15*).

HOSTS

The hosts of this disease are tomato and pepper. Foliage infection has been obtained on potato. Bacterial spot has been found on the following tomato varieties in the field: Yellow Plum, Greater Baltimore, Stone, Century, Arlington, Norton, Marvel, and Columbia. Foliage inoculation has been successful on all tomato varieties tested. This list comprises the following varieties: Bloomsdale, Magnus, Paragon, Landreth, Delaware Beauty, John Baer, Hummer, Improved Trophy, Coreless, Success, Red

[1]Contribution from the Botanical Department of Purdue University Agricultural Experiment Station, LaFayette, Ind.

The writers wish to acknowledge their indebtedness to Prof. H. S. Jackson for helpful suggestions received throughout the course of this investigation.

[2] Reference is made by number (italic) to "Literature cited," p. 155–156.

Rock, Bonny Best, Mississippi Girl, Globe, Chalk's Early Jewel, Matchless, Favorite, Comet, Golden Queen, Acme, Perfection, Buckeye State, Earliana, June Pink, Ponderosa, Early Detroit, Improved Dwarf Champion, Yellow Pear-shaped, Red Pear-shaped, Dwarf Yellow Prince, Yellow Plum, Golden Ball, Yellow Peach, and Red Cherry.

HISTORY AND OCCURRENCE

This disease as it occurs on tomato fruit has been known for several years. Coons and Nelson (*4, p. 48*) and later Coons (*3, p. 446*) briefly describe this disease as tomato canker. Nelson first found the disease at the loading station at Milan, Tenn., in 1917, and later, in August of the same year, at the loading station at Anna, Ill. In the same year he found it also at two points in Michigan, and in 1918 he noted the disease in community gardens at East Lansing, Mich. McCubbin (*9, p. 15*) reports in 1918 what appears from his illustration to be bacterial spot from fields near Walkerville, Ont., and in 1919 Nelson noted the disease on Canada tomatoes on exhibition at a convention in Detroit. Specimens were received from Muscatine, Iowa, in 1918 by the senior author.

Huelsen found bacterial spot prevalent in the Indiana canning crop near Indianapolis in 1918. In 1919 the disease was first found in an Indianapolis market garden August 10. It was found in a garden at LaFayette and in the canning crop about Frankfort and Indianapolis. It was first noticed in the canning crop August 14 and was reported August 16 by an Indianapolis grower as conspicuous on the green fruit. The disease became very conspicuous during late September. Leaf lesions were first recognized September 25. The disease was much worse in certain fields than in others. Observations were made about Paoli, Ind., and Grass Lake, Mich., but the disease was not found. The impression was gained that the disease was most prevalent in central Indiana, especially near Indianapolis.

In May, 1920, bacterial spot was found in fields of tomato seedlings near Tifton, Ga., which were being grown for shipment to northern canners. In June, 1920, the disease was found in plant beds at Campbellsburg, Ind., by H. D. Brown. July 13, 1920, the disease was found very prevalent on the foliage in a field near Kokomo, Ind., which was planted with plants grown in Georgia. Link and Ramsay of the United States Bureau of Markets found bacterial spot very prevalent on tomatoes from Dania, Fla., during the spring of 1920.

Careful search of the earlier literature has not revealed any unquestionable records of the occurrence of this disease.[1] It is, of course, not to

[1] Since this manuscript was submitted for publication, a popular account of investigations on a tomato canker (DOIDGE, E. M. A TOMATO CANKER. *In* Jour. Dept. Agr. Union So. Africa, v. 1, no. 8, p. 728–731, illus. 1920) at Pretoria, Transvaal Colony, has appeared. Very evidently the disease studied by Miss Doidge, which has been present there since 1914, is the bacterial spot of tomato. She has given an accurate description of the symptoms on leaf, stem, and fruit and states that the yellow bacterium which causes the disease will be described elsewhere under the name *Bacterium vesicatorium*, n. sp.

be confused in any way with the two vascular bacterial diseases described by Smith (*17, p. 161, 174*). Nor is it similar to the "stripe" disease of tomatoes recently described by Paine and Bewley (*10*) in England and attributed to *Bacillus lathyri*.

Many workers have found bacteria associated with blossom-end rot which we now recognize as a nonparasitic disease. Prillieux (*14, p. 19*) in 1895 termed blossom-end rot a bacterial disease. In 1898 Earle (*5, p. 19*) studied blossom-end rot in the field crop in Alabama and attributed it to bacteria which he isolated. He was of the opinion that insects carried the infection. About the same time Stuart (*18*) met with what was apparently blossom-end rot in Indiana on greenhouse tomatoes and attributed it to bacteria which he isolated. He states, however, that the lesions were not invariably at the blossom end. Miss Smith (*16*) in 1907 also found bacteria associated with blossom-end rot in Massachusetts.

In 1910 Pavarino (*11*) in Italy described blossom-end rot and a species of bacteria which he isolated. He states, however, that the disease was not confined to the fruit but was present on other parts of the plant, and he claims to have reproduced these symptoms by wound inoculation. His illustrations do not resemble bacterial spot, but his organism rather closely resembles the form causing bacterial spot, and it is of course possible that he was working with a combination of blossom-end rot and bacterial spot, since both diseases might have been present in the fields.

Groenewege (*6*) in Holland, two years later, also ascribed blossom-end rot to a species of bacteria which he isolated and described. He was able to secure inoculation upon ripening fruit by means of wounds but not upon green fruit nor upon other parts of the plant. It is not at all likely that he was dealing with bacterial spot, even though his organism somewhat resembles the form causing that disease.

Coons and Nelson (*3, 4*) in Michigan were the first to determine the nature of the bacterial spot disease which, however, they knew as canker. They isolated a yellow bacterial organism from fruit lesions in 1917 and in August of that year secured infection by means of needle prick inoculation of fruits on a caged plant in the field. In April, 1918, they also secured infection of bagged fruit in the greenhouse but did not obtain any foliage infection on sprayed plants. Only green fruits were inoculated. These workers, therefore, had the causal organism in culture and proved its pathogenicity. Unfortunately, their cultures died and it has been impossible to make a comparison with those used in the present studies.

RELATION TO BACTERIAL SPOT OF PEPPER

Assuming, upon the strength of successful reciprocal cross inoculations, that bacterial spot of pepper is caused by the same organism, we include a brief account of that disease.

A bacterial leafspot of pepper was first reported by Heald and Wolf (*7, p. 42*) from Texas in 1912. The causal bacteria were not described. In the same year a bacterial leafspot and blight of peppers was found by Jackson (*8, p. 274*) in Oregon. While the bacteria isolated were not yellow, yet the colonies somewhat resemble those of the tomato organism.

In 1917 Sherbakoff (*15, p. 79R*) worked with a bacterial spot of pepper in Florida which is very likely due to the same organism as bacterial spot of tomato. He isolated and proved the pathogenicity of a yellow organism which, however, he did not describe.

In 1918 the senior author noted the fruit lesions of what appeared to be pepper bacterial spot on southern-grown fruit in the Chicago market. In 1920 Link and Ramsay isolated a yellow organism from this so-called pepper scab and suggested that the disease was identical with the tomato bacterial spot. From a Florida-grown pepper fruit secured in the Chicago market by Link and Ramsay, which showed typical scab lesions similar to those illustrated by Sherbakoff, the authors have isolated a yellow organism resembling in culture the tomato bacterial spot organism. Inoculation of tomato plants with this organism gave typical leaf lesions, and inoculation of pepper plants with one of our tomato strains has resulted in leaf lesions resembling those described and illustrated by Sherbakoff.

Therefore, it seems safe to assume that the causal organism of pepper bacterial spot is identical with that of tomato bacterial spot. However, since we have not worked out the cultural characters of the organism isolated from pepper and since we have not found the pepper disease occuring naturally in Indiana, except in an experimental field, we shall confine our attention in this work very largely to the disease as it occurs on tomatoes.

SYMPTOMS

On the tomato fruit the disease causes scab-like lesions which may occur at any point on the surface (Pl. 24, B). These first appear as blackened, raised points or dots surrounded by a water-soaked border. Older lesions assume the form of black, slightly raised, superficial spots with a lobed or dendritic margin and a water-soaked border or halo (Pl. 24, A). From 1 to 2 mm. in diameter these lesions may enlarge until a diameter of 6 to 8 mm. is attained (Pl. 24, E). The central portions of these lesions, composed of disintegrated mesocarp tissue, soon collapses and sinks, and its surface becomes fibrous and rough. In the larger lesions the water-soaked margin is absent and the centers may become so sharply sunken that the lesion resembles a circular dry pit or crater with the ruptured epidermis about its margin (Pl. 24, D). Such lesions have apparently ceased to enlarge. Absence of the water-soaked border may be taken as evidence that peripheral enlargement has ceased, and it is apparent that fruit lesions may be checked at any stage of their development. Actively enlarging lesions are not found on mature fruit.

The lesions frequently penetrate almost through the pericarp but apparently not into the juicy interior of the fruit. Coalescence of these lesions may cause very large blotchlike areas of disorganized pericarp tissue (Pl. 24, C). As a general rule, no marked malformation of the fruit is produced.

Associated with these lesions are often found small, circular, whitish "bird's-eye" spots about 2 mm. in diameter with a dark raised central point or a lenticular rift at the center and a cavity under the white area (Pl. 24, D). Cases were found in which the blackened scabs of the bacterial spot had developed from the dark point at the center of the white spot and the white halo was less conspicuous. Preliminary attempts to isolate the bacterial spot organism from these white spots have been unsuccessful, and such lesions have never resulted from artificial inoculation. It has been assumed that these white "bird's-eye" spots are a form of insect wound through which infection may occur.

Needle-prick inoculation of fruit in the greenhouse has resulted in two types of lesions. Both steel needles and very fine capillary glass points have been used. In cases in which the needle was dipped in the inoculum and then inserted into the fruit, no superficial lesion resulted, but a rather firm blackish core of infected tissue was produced in the mesocarp well beneath the epidermis, resembling the apple bitter-pit type of lesion. When, however, the inoculum was applied to the surface of the fruit after the needle wounds were made, the lesions rather closely resembled those occurring in the field, except that the lesions were brown instead of black and caused more of a depression by their inhibition of fruit growth (Pl. 24, F).

Bacterial spot lesions on the fruit may be differentiated from nailhead spot caused by Macrosporium by their black or darker brown center, irregular margin, water-soaked border or halo, deeper penetration, and greater disintegration of the central tissues. Spots resulting from wound inoculation with *Septoria lycopersici* in the fruit also differed distinctly from the bacterial spot.

On leaves of mature plants in the field, this disease causes small black spots with a slight tendency toward an angular outline (Pl. 25, E). These spots may be greasy on the upper surface. The center may be at first translucent and the margin black. The central tissue soon becomes black and parchmentlike with a tendency to crack at the center. About the black center of such lesions, as examined in transmitted light, there is a distinct, yellowish, translucent margin. Lesions are more abundant on young leaves and often are confined to one or two leaflets of a leaf and to certain regions on each leaflet such as the basal portion or a lateral half.

Among the seedlings in the fields noted in Georgia, this disease caused a conspicuous spotting of the leaves. There was in many cases a yellow discoloration of the tissue about the lesions, and badly spotted leaves were distinctly yellowish. Such leaves dropped off very readily. This

especially destructive effect of the disease among seedlings has also been noted in the greenhouse.

The leaf lesions are not easily differentiated from Septoria lesions, and it is likely that leaf infection has not been previously detected because of its similarity to Septoria leafspot. Bacterial spot can be recognized, however, by its jet black center, greasy upper surface, less circular outline, sharp translucent margin, and lack of pycnidia. There is more abundant infection of young foliage from bacterial spot.

A somewhat different type of leaf infection has been secured by atomizer inoculation under greenhouse conditions. Such lesions first appear as small, circular, slightly water-soaked spots, 1 to 2 mm. in diameter. These are at first visible only on one leaf surface and are very inconspicuous except as viewed in transmitted light, in which case they appear translucent. These lesions soon become visible on both leaf surfaces as circular, water-soaked or blackish, translucent, greasy spots which remain small on old leaves (Pl. 26, D). On young leaves such lesions enlarge to irregularly circular, dark brown to black spots of a diameter of 3 to 4 mm. with yellowish or translucent margins (Pl. 26, C). Under greenhouse conditions the lesions exhibit no tendency to be delimited by veins.

In the early stages of a leaf lesion under very humid conditions in the greenhouse the lower epidermis is puffed up by the bacterial mass underneath. A greasy exudate is formed on both upper and lower surfaces, and occasionally amber droplets of exudate are formed on the lower surface. Later the central tissue collapses, and on the old leaves the spots appear sharply sunken on the lower epidermis and under a binocular microscope resemble minute circular pits or depressions with fimbriate margins. About the margin there is a black lacelike discoloration in the tissue due to the intercellular advance invasion. In large lesions on younger leaves the centers become dry and parchmentlike and show a very distinct dark lacelike pattern when viewed in transmitted light. These parchmentlike centers are usually brown or tan colored, translucent, and have dark, irregularly circular margins (Pl. 25, A; 26, B). Such lesions tend to crack at the center.

Thick-set incipient infection resulting from atomizer inoculation may produce large yellow patches on old leaves which become dry, dead areas. Coalescence of lesions may cause the disfiguration and death of young leaflets (Pl. 26, A). Infection of very young, growing parts also causes extreme distortion, especially infection of the rachis, petiolule, and leaf margin (Pl. 26, A).

Cotyledon lesions are usually very small, craterlike pits or depressions, with an irregular margin, and are often gray or silvery in color. As long as a cotyledon remains green a lesion is apt to be visible on only one surface, although lesions may occur on either surface. When the cotyledon becomes older the lesions become visible from the other surface as dark, lead-colored spots. Badly infected cotyledons turn

yellow prematurely. Cotyledon lesions resulting from seed inoculation are larger, black or leaden gray, shiny, depressed areas, irregular in outline, and often cause distortion (Pl. 25, B, C). These are likely to occur near the tip where the seed coat adhered and was carried up after germination.

Stem, petiole, and rachis infection is of common occurrence. Such lesions are circular to linear, blackened, at first slightly elevated spots with a very irregular margin, and are 3 to 5 mm. long, often considerably longer (Pl. 25, D).

Petiolule and rachis lesions may cause the death of leaflets, and secondary infection of seedlings is particularly destructive. Stem lesions are not in themselves a serious injury to the plant except near the growing points. Peduncle and hypocotyl lesions have been produced.

Inoculation of pepper foliage has yielded circular water-soaked lesions similar to those described by Sherbakoff. Fruit and leaf lesions of the disease on peppers are well described and illustrated by Sherbakoff.

Inoculation of potato foliage has resulted in small, sunken, black dots on the lower surfaces of the leaves.

ECONOMIC IMPORTANCE

Under field conditions, as observed in 1919, bacterial spot is apparently a minor disease of the foliage of mature plants, but its attack upon the fruit is very objectionable from both the market and the canning standpoints. The lesions are unsightly and if numerous and of the larger type destroy much of the edible pericarp, thus rendering a fruit unsalable (Pl. 24, C, D). In addition, these lesions afford entry to rot-producing organisms.

Canners object to this disease because even the rather superficial lesions are not removed with the skin but remain on the peeled fruit. Affected fruits must therefore be culled out of the first-grade canning stock and either discarded or used for catsup. As the result of a chance observation made at the sorting aprons in a canning factory, September 25, while tomatoes grown near Indianapolis were passing over the belt carrier, it was estimated that about 5 per cent of the fruits were affected with bacterial spot. In fact, growers and canners in general are coming to recognize this disease as a real source of loss. Huelsen reports that in 1918 a great many tomatoes were thrown out at this factory because of bacterial spot, and as a result of his field work in the canning crop during that season, he considers bacterial spot, as it occurs on the fruit, a serious disease. Nelson found the disease of serious importance in Tennessee and southern Illinois in 1917. He found it in all Illinois fields examined.

In one out of eight fields examined in southern Georgia where seedling transplants were being raised for the use of northern growers, bacterial

spot was found to be epidemic and serious. Not only were the leaves badly spotted and the plants rendered somewhat objectionable to the more observant consignees, but among the plantings badly stunted by other unfavorable growing conditions, the severe attack of this disease on the foliage was particularly destructive. Later, H. D. Brown found bacterial spot of considerable importance in tomato plant beds at Campbellsburg, Ind. Definite centers of infection were noted. It is evident, therefore, that this disease may be classed among the major diseases of seedling tomatoes. Severe foliage infection causing the death of many leaflets was noted on southern-grown plants in a field near Kokomo, Ind., in the middle of July. In a warm, wet season, bacterial spot may therefore be destructive as a foliage disease in the field crop.

Link and Ramsay report that bacterial spot equaled nailhead in severity and prevalence on tomatoes from Dania, Fla., in 1920.

Sherbakoff found the disease of peppers to be of considerable importance as a blemish of the fruits. Observations in the Chicago market bear out this opinion.

CAUSAL ORGANISM

ISOLATION

The first series of isolations from tomato fruit lesions were made in the following manner: The surface of the fruit was carefully wiped off with mercuric chlorid 1 to 1,000, and the epidermis over a lesion was sliced off with a flamed scalpel. Portions of the underlying blackened tissue were cut out with a flamed scalpel and planted in a poured plate of potato agar. Black lesions in various stages of development were used. Out of 80 tissue plantings made, only 4 developed fungi, while 73 yielded bacterial growth of a more or less uniform type. By plating out from these colonies about the tissue transfers, the organism which later proved pathogenic was isolated.

When it thus became apparent that no fungus was associated with the black fruit lesions, isolations were made by slicing off the epidermis, macerating some of the underlying infected tissue in a drop of sterile water on a flamed slide, and plating out from this water in potato agar by the loop dilution method.

Stem and petiole lesions were rinsed in mercuric chlorid 1 to 1,000, excised with a flamed scalpel, rinsed in sterile water, and then macerated in drops of sterile water on a flamed slide. A loopful from each of these was transferred to a second drop of sterile water, which was then plated out in potato agar by the loop dilution method.

Leaf lesions were cut out with a flamed scissors, immersed in mercuric chlorid 1 to 1,000 a few minutes, rinsed in sterile water, and macerated in drops of sterile water, from which plates were poured as described above.

Isolations were made from material collected at Indianapolis, Frankfort, and La Fayette. From all of these sources and all types of lesions the same type of yellow, translucent, rapidly growing bacterial colony was obtained. Numerous strains were isolated by transfer to agar slants, and all exhibited similar cultural characteristics. The pathogenicity of a number of these strains was proved by numerous successful inoculations in the greenhouse with subsequent reisolation and in many cases reinoculation. These tests are discussed on a succeeding page. Strains used to any extent were tested for freedom from contamination by poured plates. For the detailed morphological and physiological study, two strains were used, one isolated from a fruit collected at La-Fayette, the other from a leaf collected at Indianapolis. These were proved pathogenic by repeated inoculation and reisolation, and their purity was proved by poured plates. No marked differences were noted. The strain isolated from pepper fruit has not yet been carefully studied.

MORPHOLOGY

The organism is a medium-sized rod with rounded ends, usually occurring singly or paired, occasionally in short chains. The cells stain readily with Ziehl's carbol fuchsin, anilin gentian violet, and Loeffler's methylene blue. Frequently two heavily staining portions are visible, one at each end of the rod. Age of the culture does not appear to affect the size measurements. When stained with Loeffler's or Van Ermengem's flagella stains, the cells vary in width from 0.65 to 1.35 μ and in length from 1.44 to 2.79 μ, with an average of 0.85 by 1.94 μ, as measured with a vernier micrometer.

The organism is motile by means of one polar flagellum (Pl. 28, B). The flagella were stained by Morrey's modification of Loeffler's method and more readily by Van Ermengem's method, in which, by necessity, pyrogallic acid was substituted for gallic acid in the formula. Flagella were found in both old and young cultures. The best stains were obtained from 24-hour beef agar slant cultures.

Endospores and involution forms have not been noted. It has not been possible to demonstrate the presence of capsules by the Welch or Hiss methods in stains made from beef, potato, or blood agar. However, in greatly enlarged photomicrographs made from Van Ermengem flagella stains, a narrow but distinct clear zone or halo about each rod indicates the presence of a thin capsule. The organism is Gram-negative.

CULTURAL CHARACTERS

The organism grows very readily on a wide variety of culture media. For general laboratory use a 2 per cent potato agar with 2 per cent peptone was found most satisfactory. In the following studies cultures were incubated at 25° C. unless otherwise specified. The reaction of

media as expressed in Fuller's scale was adjusted by titration with phenolphthalein as an indicator, in which the first appearance of a permanent pink color was taken as the neutral point. Color determinations were made by reference to Ridgway's color standards.[1] Inoculations were made from a water suspension of the organism. A culture of *Bacillus coli* was carried in a parallel series of tests on practically all the media.

AGAR POURED PLATES.—On + 10 beef-peptone agar colonies appeared in 24 hours. In 48 hours surface colonies were 1 mm. in diameter, translucent, and slightly fluorescent. In 5 days surface colonies were 2 to 3 mm. in diameter, circular with entire margins, convex or pulvinate, smooth, glistening, translucent, and straw yellow. Submerged colonies remained very small and were lenticular in shape, except that those in contact with the bottom of the dish spread underneath the agar to form circular, grayish fluorescent spots. The agar was unchanged in color, and there was no odor.

On plain potato agar, growth was more abundant and the yellow pigment was more pronounced. In 10 days colonies were 8 mm. in diameter, circular with entire margins, pulvinate, smooth, glistening, and naphthalene yellow (Pl. 28, D). Submerged colonies remained small and lenticular or three-cornered. On potato agar with 2 per cent peptone the colonies attained a diameter of 7 mm. in 5 days. On + 5 potato soft agar (1½ per cent), colonies resulting from the planting of a 2-mm. loop of a water suspension on the agar surface attained a diameter of 40 mm. in 7 days. In the peripheral zone there were distinct radial striations of denser pigmentation, while the central zone showed a granular or netted pattern. The consistency was butyrous or gelatinous.

AGAR STABS.—On + 10 beef-peptone agar, a rather scanty filiform growth with a beaded outline occurred along the stab, more noticeably toward the top; and the surface growth, which was rather scanty at the end of 2 days, later became abundant.

On plain potato agar, scanty growth occurred along the upper 5 to 10 mm. during the first 2 days and later was visible along the upper 15 mm., but not at the lower end of the stab. The surface growth in 2 days was abundant, convex, smooth, glistening, and naphthalene yellow. After 18 days the entire surface was covered 5 mm. deep.

AGAR SLANT.—On + 10 beef-peptone agar, the growth was abundant, at first filiform, later spreading with an entire margin, raised, smooth, glistening, translucent, at 2 days massicot yellow, at 5 days mustard yellow, and at 19 days anilin yellow. Along the edge of the culture there were short parallel striations of denser color perpendicular to the margin. The agar remained unchanged in color, and there was no odor.

On plain potato agar the growth was more vigorous and in 5 days was

[1] RIDGEWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington D. C., 1912.

abundant, spreading with an entire margin, convex, smooth, glistening, and translucent. At 2 days the color was marguerite yellow, and at 5 days it was naphthalene yellow with short parallel striations of denser color perpendicular to the margin and a granular or netted pattern through the center. At 9 days the color was a straw to amber yellow, and the surface of the agar was covered by the profuse growth. The consistency was gelatinous. The color of the agar was unchanged. There was no odor.

On plain 2 per cent dextrose agar the growth was almost as vigorous as on potato agar and at 4 days was a marguerite yellow.

GELATIN PLATES.—On gelatin plates incubated at 18° C., circular yellow colonies 3 to 5 mm. in diameter were visible in 5 days. Liquefaction was at once evident as saucer- or cup-shaped cavities under the colonies, and very soon the entire contents of the plates were liquefied.

GELATIN SLABS.—In stab cultures held at 18° C., scanty growth at the surface and a filiform growth along the upper half of the stab were visible in 3 days. In 4 days liquefaction was evident in a cup-shaped cavity on the surface. The subsequent liquefaction was of the stratiform type, progressing slowly downward until in a month the liquefied portion was 2 to 3 cm. deep. There was a yellow precipitate at the bottom of the liquefied portion. No change occurred in the color of the gelatin.

POTATO CYLINDERS.—On steamed potato cylinders growth was more profuse than on any other culture medium. In 2 days the growth was abundant, spreading, raised, smooth, glistening, and butyrous or gelatinous in consistency, and a naphthalene yellow in color. In 5 days the color had deepened to a straw yellow and later became amber yellow. In 10 days the cylinder was completely immersed in the gelatinous bacterial growth. The color of the potato tissue was not altered.

On sterile uncooked potato cylinders growth appeared in 2 days and was abundant in 4 days but was checked by drying of the medium. A conspicuous channel, lined with blackened slime, marked the path of the needle stroke. Microscopic examination of the disintegrated tissue showed the cells separated to some extent by middle lamella solution and the cell walls brown in color. On sterile green tomato disks similarly inoculated a blackened channel was also formed, but the subsequent development was much more profuse. There was no bad odor produced. The growth was abundant, spreading, smooth, glistening, and yellowish. A shallow layer of the underlying tissue was softened and blackened, and the tissue was darkened and water-soaked in advance of the rot. Microscopic examination of the affected tissue showed the cells separated to a limited extent as if by middle lamella solution.

MILK.—A slow clearing without coagulation occurred in milk cultures. In 5 days a yellowish surface film was formed, and a slight clearing was visible near the surface. In 10 days the entire culture was translucent

and in 20 days the liquid was semitransparent with a yellow precipitate in the bottom of the tube. The consistency of the liquid was unchanged.

LITMUS MILK.—Lavender-colored litmus milk was completely decolorized in 10 days, but no pink color was produced. Therefore no acidity was produced. In 20 days the blue color reappeared in the bottom of the tube, but the upper part of the liquid remained amber-colored. Clearing caused by digestion took place as described above, and a yellow surface film and precipitate were formed.

BROM CRESOL PURPLE IN MILK.—In accordance with modern bacteriological technic (*2*), the litmus milk tests were supplemented by the use of milk containing brom cresol purple in a concentration of 0.0016 per cent, which gives a distinct bluish color. This indicator changes from its alkaline purple color at a hydrogen-ion concentration of P_H 6.8 to its acid yellow color at P_H 5.2 and affords a more reliable index of the changes in the true acidity of milk than does litmus. Twelve days after inoculation there was no change in color, but in 16 days the inoculated tubes were a deeper blue than the controls. At no stage did the color become lighter. The hydrogen-ion concentration in milk was therefore lowered by the growth of this organism.

METHYLENE BLUE IN MILK.—Reduction of methylene blue ocurred. Decolorization began in 2 days, and after 5 days all the liquid except a surface layer 5 mm. deep was completely decolorized. At the end of 20 days the upper layer of 20 mm. in depth was a light grass-green. Digestion of the milk occurred as previously noted, and a yellow precipitate was formed.

REDUCTION OF NITRATES.—In fermentation tubes containing 1 per cent potassium nitrate bouillon, a light growth occurred in the open arm, but there was no growth in the closed arm. No gas was formed. Tests with Trommsdorf's reagent indicated that there was no nitrite formed. Additional tests were made with test tube cultures in 2 per cent Difco peptone water containing 1 per cent potassium nitrate. Good growth occurred, but no test for nitrites nor ammonia was secured at 4, 6, and 11 day intervals. Therefore nitrates were not reduced.

CARBON METABOLISM.—For fermentation tubes, a 2 per cent peptone solution was used as the base for six solutions made by adding 2 per cent of the following carbon compounds: dextrose, saccharose, maltose, lactose, glycerin, and mannit. Cultures were run in duplicate and incubated at 22° C. In 3 days there was heavy clouding in the open arm of each tube and no growth whatever in the closed arm. There was a sharp plane of separation between the clouded and the clear medium. In 10 days a yellow surface pellicle and yellow precipitate had formed in the open arm in each case. The plane of separation mentioned above was not quite so sharp, and in some cases where the connecting tube was of greater diameter a slight clouding was noted in the closed arm. No gas was produced in any of these tubes, whereas in the *Bacillus coli*

cultures heavy growth in the closed arm and abundant gas formation had occurred.

After 40 days, titration with phenolphthalein showed that there was very little change in acidity. The saccharose and mannit cultures were slightly more acid than their controls, the maltose culture was unchanged, the dextrose and glycerin cultures were slightly less acid than their controls, and the lactose culture was quite decidedly less acid. All the *Bacillus coli* cultures showed greatly increased acidity.

To determine more accurately the changes in true acidity in these media, recourse was had to the sulphone-phthalein indicators as advocated by the Society of American Bacteriologists (*2*). Three series of four test tubes each of the six media described above were prepared. One series contained brom cresol purple, one contained brom thymol blue, and the third contained phenol red. The indicators were used in a concentration of 0.0016 per cent. The characteristics of the three indicators are presented in Table I.

TABLE I.—*Changes in acidity as shown by sulphone-phthalein indicators*

Indicator.	Full acid color.	Full alkaline color.	Sensitive range.
			Ph.
Brom cresol purple	Yellow	Purple	5.2 to 6.8
Brom thymol blue	do	Blue	6.0 to 7.6
Phenol red	do	Red	6.8 to 8.4

Thus these indicators should serve for P_H values between 8.4 and 5.2, but our standards made up according to Sörensen[1] showed that the usable range was more restricted. The media were adjusted to a slightly alkaline reaction of about P_H 7.5 as indicated by the blue shade of brom thymol blue and the yellowish red with phenol red. This gave a full alkaline purple with brom cresol purple. These tubes were sterilized, and two of each set of four were inoculated with the bacterial spot organism, one was inoculated with *Bacillus coli*, and one tube was held as a control. All were incubated at 22° C.

Vigorous growth occurred in all the cultures. A surface pellicle and precipitate were formed in every case. Observations were made at the end of 2 days, 7 days, 14 days, and 43 days. In all the cultures of the tomato organism containing brom cresol purple and brom thymol blue there was no change in color, while in the cultures containing phenol red a brighter red color appeared within 14 days and all were uniformly brighter red at the end of 43 days. The lactose cultures were the first to show this red. All the *Bacillus coli* cultures became yellow. The controls remained unchanged.

[1] SÖRENSEN, S. P. L. ÜBER DIE MESSUNG UND BEDEUTUNG DER WASSERSTOFFIONEN-KONZENTRATION BEI BIOLOGISCHEN PROZESSEN. *In* Ergeb. Physiol., Jahrg. 12, p. 393-532, 12 fig. 1912. Literature, p. 394-398.

Since none of the cultures of the tomato organism showed any change toward the yellow color, it is evident that in no case was there any increase in the hydrogen-ion concentration. On the contrary, as indicated by the phenol red, there was a decrease in the hydrogen-ion concentration to some point in the neighborhood of P_H 8. This decrease occurred most rapidly in the lactose cultures.

That this failure to produce acid was not due to the possible masking effect of the alkaline products of peptone digestion was proved by the fact that in a bouillon culture containing 5 per cent peptone there was after 3 days' incubation no decrease of the hydrogen-ion concentration as indicated with phenol red.

AGAR WITH SUGARS.—Abundant growth occurred in slant cultures on litmus dextrose, litmus maltose, and litmus lactose agar. There was no change in the litmus lactose agar until after 20 days, when the color became more bluish. In the other two media the litmus was entirely faded at the end of 10 days because of reduction, but a pale blue color returned at 20 days similar to that in the lactose agar. No pink color was produced at any time. This indicates that there was no increase in acidity. A pink color was produced in a parallel series of *Bacillus coli* cultures.

To check up the test with litmus, a similar test was run in a triple series with the three sulphone-phthalein indicators described above—that is, brom cresol purple, brom thymol blue, and phenol red. These media were adjusted to a reaction very close to true neutrality or P_H 7 as indicated by the grass-green color of the brom thymol blue and the yellow color of the phenol red. Abundant growth occurred. While the *Bacillus coli* cultures became distinctly yellow, there was no marked change in the other cultures. The phenol red assumed a more distinct red than the controls. The brom thymol blue in the dextrose agar and the brom cresol purple in the dextrose and maltose agars faded slightly. With the tomato organism, therefore, no increase in acidity occurred in these sugar agars.

ACTION ON STARCH.—In plates of beef agar to which potato starch was added, halos 3 to 6 mm. in width appeared about the colonies from loop plantings at the end of 5 days. When a solution of iodin in potassium iodid was added, the clear zones under and around each colony proved that the starch had been destroyed therein. In similar plate cultures on agar containing corn starch, clear halos were also visible when the iodin test was applied. There was, therefore, vigorous diastatic action on potato and corn starch by an enzym which diffused out into the agar.

The cooked potato tissue used as a culture medium was tested with iodin. Microscopic examination showed that the tissue disintegrated by the growth of the organism gave no blue color, even within the cells, while in the uninoculated tissue a deep blue color developed. In the

cultures on uncooked potato cylinders, starch was still present in the disintegrated tissue. There was, therefore, strong diastatic action on the starch in cooked potato tissue.

ACTION ON CELLULOSE.—No evidence of any dissolving action on cellulose was secured by Söhngen's method,[1] in which filter paper is blackened by dipping in manganese sulphate and potassium permanganate, sterilized, and moistened with nutrient media previous to inoculation. Cytolytic action would be evidenced by the disappearance of the black manganic oxid.

TESTS FOR INDOL AND AMMONIA.—Cultures in beef-peptone bouillon, 6 days old, gave no test for indol when tested with potassium nitrite and sulphuric acid. Similar cultures of *Bacillus coli* gave a positive test for indol. Cultures in 5 per cent peptone bouillon yielded no test for indol after 5 days' incubation at 22° C. and no test for ammonia with Nessler's reagent.

No growth occurred in Fermi's, Uschinsky's, and Cohn's solutions.

BLOOD SERUM.—Stroke cultures on plain solidified blood serum after 3 days showed abundant growth, spreading, raised, smooth, and glistening. Liquefaction had begun, and at 9 days a channel was formed along the stroke and the bacterial growth was yellow.

On slants of Loeffler's blood serum, the growth in 3 days was abundant, spreading, flat, smooth, glistening, and yellow in color. No liquefaction occurred, and the subsequent growth was not as vigorous as on plain blood serum.

TOLERATION OF SODIUM CHLORID.—Tubes of neutral beef-peptone bouillon containing 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 per cent of pure sodium chlorid were used in this test. Good growth occurred in the presence of 0.5 per cent and 1 per cent sodium chlorid, but not in any of the higher percentages. *Bacillus coli* tolerated as high as 5 per cent.

TOLERATION OF ACIDITY.—Tubes of beef-peptone bouillon titrating +30, +25, +22, +20, +18, +15, +14, +12, +10, +5, 0, −5, −10, −20, −25, −30, and −40 were prepared. These media were adjusted by the use of normal hydrochloric acid and normal sodium hydroxid. Growth occurred only in the +10, +5, 0, −5, and −10 tubes and was most vigorous in the +5 broth and least vigorous in the −5 and −10 broth. The upper limit of tolerance lies, therefore, between +10 and +12 and the lower limit between −10 and −20. *Bacillus coli* grew at +20 and −30.

That the acidity in the +10 and +5 cultures was neutralized by the organism was proved by adding a few drops of the indicator phenol red. This remained red, proving that the P_H value had been lowered to a point below 7.7, while the control as tested with methyl red had a P_H value of about 5.8.

[1] SÖHNGEN, N. L. UMWANDLUNGEN VON MANGANVERBINDUNGEN UNTER DEM EINFLUSS MICROBIOLOGISCHER PROZESSE. *In* Centbl. Bakt. Abt. 2, Bd. 40, No. 22/25, p. 545–554, 3 pl. 1914.

To determine the tolerance of true acidity as indicated by the hydrogen-ion concentration, duplicate series of tubes of beef-peptone bouillon were adjusted with normal hydrochloric acid to the P_H values presented below by the comparison of the indicator methyl red in the tubes with a set of standards. For the P_H 6.4 series, brom cresol purple was used. After inoculation these tubes were incubated at 22° C. The results are presented in Table II.

TABLE II.—*Tolerance of acidity as indicated by growth in media at various hydrogen-ion concentrations, incubated at 22° C.*

Incubation period.	P_H 6.4.	P_H 5.8.	P_H 5.6.	P_H 5.4.	P_H 5.3.
3 days....	Heavy growth	Light growth.	No growth....	No growth....	No growth.
5 days....	.....do.....	.....do.....	Light growth.	Light growth.	Do.

The tubes of P_H 5.3 were then heavily reinoculated from a slant culture, and in 3 more days growth was evident. The acid range was then extended and the test repeated. This set of cultures was incubated at 25° C. The results are presented in Table III.

TABLE III.—*Tolerance of acidity as indicated by growth in media at various hydrogen-ion concentrations, incubated at 25° C.*

Incubation period.	P_H 5.4.	P_H 5.3.	P_H 5.2.	P_H 5.	P_H 4.8.	P_H 4.7.	P_H 4.6.
3 days..	No growth...	No growth...	No growth.	No growth.	No growth.	No growth.	No growth.
4 days..	Light growth.	Light growth.	.....do.....	.....do.....	.....do.....	.....do.....	Do.
12 days.	Heavy growth	Heavy growth	.....do.....	.....do.....	.....do.....	.....do.....	Do.

At the end of 12 days the tubes in which no growth had occurred were reinoculated from one of the cultures that grew at P_H 5.3. In 7 days growth had occurred in both the P_H 5.2 tubes and in one of the two P_H 5 tubes, but in none of the others. Therefore, the upper limit of tolerance of the hydrogen-ion concentration may be taken as P_H 5.3, although this was increased to P_H 5.2 and P_H 5 by reinoculation from cultures growing in media of P_H 5.3. Development is noticeably checked by a P_H value of 5.6, and P_H 6.4 is more favorable than P_H 5.8.

TEMPERATURE RELATIONS

The organism grows well in a wide range of temperature. Slant and plate cultures on potato agar, inoculated by strokes made from a water suspension of the bacteria and incubated in moist chambers at 10°, 15°, 18°, 22°, 25°, 30°, and 35° C., proved that the organism was able to develop at all these temperatures. At 10° the growth was very slow, at 15° it was more abundant, and at 18° fairly good growth occurred. The

development at 35 was not much better than at 15°. The most vigorous development occurred at 22°, 25°, and 30°; and although there was very little noticeable difference, the optimum temperature seems to lie between 25° and 30°. High temperatures in the greenhouse noticeably shorten the incubation period.

An interesting correlation between temperature and pigmentation was noted. In the cooler temperatures the yellow color was more intense than in the higher temperatures, ranging from a barium yellow at 10° C. to a naphthaline yellow at 22° and a marguerite yellow at 30° and 35°. At 35° the colonies soon became almost colorless and translucent.

In determining the thermal death point, water suspensions from agar slant cultures were subjected to 10-minute exposures to a series of temperatures in a water bath and tested by loop transfers to agar slants. As a result of two trials it was determined that the thermal death point lies between 49° and 50° C.

EFFECT OF SUNLIGHT

The organism was found to be very susceptible to sunlight. Practically complete sterilization of the unshaded portions of agar poured plates was obtained by 20, 30, and 40 minute exposures to morning sunlight. When a black background was used, colonies developed in the shaded portions of the plates, but when a white background was used, colonies failed to develop even in the shaded portions of plates exposed 40 and 60 minutes. This indicates that the organisms are killed by reflected white light as well as by direct sunlight.

RESISTANCE TO DESICCATION

The organism is very resistant to desiccation. When dried on sterile cover glasses it was found alive after 25 days. In this test a loopful of a water suspension of the bacteria from an agar culture was allowed to dry on each cover glass. Tests were made by inserting the cover slips into tubes of slanted agar.

To determine the resistance to desiccation on tomato seed, a water suspension of the bacteria was poured over sterilized seed in Petri dishes and allowed to dry. This seed was tested at intervals by planting in agar plates. In a test as yet unfinished the organism was still viable after 11 months' desiccation. As will be discussed later, it has already been proved that the organism lives over winter on the seed.

SUSCEPTIBILITY TO GERMICIDES

In a water suspension of the bacteria, complete sterilization was obtained by 5-minute exposures to mercuric chlorid in concentrations of 1 to 1,000, 1 to 2,000, and 1 to 4,000, and by 5-minute exposures to 10 per cent, 5 per cent, and 2 per cent commercial formaldehyde.

TAXONOMY

As has been previously pointed out, many workers have found bacteria associated with tomato diseases, particularly blossom-end rot. The bacteria isolated by Earle (*5*), by Stuart (*18*), and by Miss Smith (*16*) from blossom-end rot are not named nor are they adequately described. They show no similarity with the form causing bacterial spot.

The bacteriosis of tomato described in 1910 by Pavarino (*11*) is of considerable interest. While the fruit lesions are very evidently blossom-end rot, stem and foliage lesions are also present. He isolated from both fruit and leaves a yellow organism with which successful wound inoculations were made on fruit, stems, and leaves. He named this organism *Bacterium briosii*. In morphology and cultural characters this closely resembles the organism causing bacterial spot except that it has 1 to 4 flagella, the agar colonies have a lobed margin, and its optimum temperature was lower. As has been previously stated, it is necessary to admit that Pavarino may have been working with a combination of blossom-end rot and bacterial spot, but the description is not sufficiently clear-cut to justify the assumption that *Bact. briosii* is identical with the organism causing bacterial spot. Pavarino and Turconi (*12*) recently have described a *Bacillus capsici* as the cause of a pepper-wilt disease in Italy. This organism differs from the tomato organism in that its colonies are whitish gray and it produces acid and coagulates milk.

The organism isolated by Groenwege (*6*) in 1912 from tomato blossom-end rot and named by him, *Phytobacter lycopersici*, rather closely resembles the organism under consideration in cultural characters. However, the flagellation is not described, and his account of mutations in his cultures suggests the presence of more than one species. In addition, infection was secured only upon the mature or ripening fruit. There are not, therefore, sufficient grounds to assume that his *P. lycopersici* is the same as the organism causing bacterial spot.

Perotti and Cristofolletti (*13*) in 1914 report from Italy a *Pseudomonas polycromigena* associated with *Cladosporium herbarum* as a secondary invader of tomato fruit. In both pathogenicity and cultural characteristics this organism is quite unlike the one causing bacterial spot.

Nelson, working with Coons (*3*), isolated from true bacterial spot a yellow organism with which fruit inoculation was successful. Recently Link and Ramsay have isolated a yellow organism from bacterial spot on Florida tomatoes, cultures of which closely resemble those of the causal organism. Sherbakoff (*15*) isolated a yellow organism from bacterial spot of pepper and proved its pathogenicity. Link and Ramsay have also isolated a yellow organism from pepper-scab, and the similarity of their cultures to those which they isolated from tomatoes led them to suspect that the pepper and tomato diseases were related.

It was upon their suggestion that the successful cross inoculations subsequently reported were made.

Since the organism known to be involved in bacterial spot of tomato has not been described and named,[1] it is given the following chacterization.

TECHNICAL DESCRIPTION

Bacterium exitiosum, n. sp.[2]

Cylindrical rods, rounded at ends, solitary or in pairs; individual rods 1.5 to 2.7 μ by 0.6 to 1.3 μ; motile by a single polar flagellum; aerobic, no spores; not conspicuously capsulated.

Superficial colonies on potato agar, round, pulvinate, smooth, glistening; naphthalene yellow, with radial striae of color in peripheral zone; margin entire.

Gelatin rapidly liquefied; no acid produced in milk; digests casein; nitrates not reduced; no acid or gas produced in media with various carbohydrates; Gram-negative.

Group number 211.3332513.

Pathogenic on *Lycopersicum esculentum* Mill, forming lesions on leaf blade, rachis, petiole, cotyledon, stem, peduncle, and green fruit. Fruit lesions destructive. Also pathogenic on leaves and fruit of *Capsicum annuum* L. and on leaves of *Solanum tuberosum L.*

Type locality: Frankfort, Ind.

Distribution: Widespread.

PATHOGENICITY

Since the fall of 1919 numerous series of inoculations upon tomatoes under greenhouse conditions were attended by uniformly successful results. Cultures from four sources were used, but most of the work was done with the two strains used in the cultural tests. Repeated reisolations have been made, and the identity of the causal organism has been verified. One reisolated strain was extensively used in cultural and inoculation tests. Reciprocal cross inoculation upon pepper and tomato plants has also been successful.

Foliage infection of tomato was readily produced by spraying plants with an atomizer containing a water suspension of the organism from agar cultures. For much of the foliage inoculation work a moist chamber was used, but it was found that ordinary greenhouse conditions also permitted infection. Infection of green fruit was obtained through puncture wounds. Cotyledon infection was effected by spraying flats of seedlings and by planting inoculated seed. The incubation period on foliage was three to six days and on fruit five to six days. Typical inoculation tests are described in the following paragraphs.

FOLIAGE INOCULATION

On January 31 two small potted tomato plants were thoroughly atomized on both upper and lower leaf surfaces with a water suspension of the organism from an agar slant culture 8 days old. These plants were

[1] See footnote reference on page 125 to the work of Miss E. M. Doidge.

[2] According to Migula's classification and Buchanan's revision the combination would be *Pseudomonas exitiosa*, n. sp. (BUCHANAN, R. E. STUDIES IN THE NOMENCLATURE AND CLASSIFICATION OF THE BACTERIA. V. SUBGROUPS AND GENERA OF THE BACTERIACEAE. *In* Jour. Bact., v. 3, no. 1, p. 48–51. 1918.)

held in an inoculation chamber in which the temperature varied from 59° to 90° F. Ten days later there were numerous lesions on both plants.

On February 12 two small tomato plants were atomized with a water suspension from a 34-day-old agar slant culture, one (A) on both leaf surfaces and one (B) on only the upper surfaces. These plants along with a third plant (C) atomized with distilled water as a control were held in an inoculation chamber. Five days later numerous incipient lesions were visible on the lower surfaces of the leaves of plant A. The next day one lesion was found on plant B. After 11 days, plant A was heavily infected, plant B showed only a few lesions and these were on rachises and young leaves, and the control plant C was free from infection. This indicated that infection occurred more readily through the lower epidermis.

A second test yielded similar results. On February 14 two plants were atomized with a water suspension of the organism, one (A) on both leaf surfaces, the other (B) on only the upper surfaces of the leaves. After three days in the inoculation chamber, a few incipient lesions were noted on plant A, and by the next day the leaves were thickly dotted with incipient lesions. No lesions were found on plant B. Nine days after inoculation the coalescence of lesions was causing the death of large leaf areas on plant A. A very few scattered lesions had appeared on the younger leaves of plant B, while the older leaves were practically free from infection.

In another test in which 16 plants were inoculated, 8 on the lower leaf surfaces only and 8 on the upper leaf surfaces, much more abundant infection was secured where the lower leaf surfaces were inoculated. These plants were removed from the moist chamber in sets of four at intervals of one day, two days, four days, and six days. The infection on the plants removed at the end of one day was not quite as heavy as on those removed after the longer intervals. Otherwise, no marked influence of the humidity factor was noted.

In all these foliage inoculations, the lesions on the young leaves became larger than on the old leaves (Pl. 26, C, D). Rachis, petiole, and stem lesions were also obtained, but no fruit infection occurred. In subsequent inoculations made during the warmer weather, incipient lesions have been detected as soon as 36 hours after inoculation.

With a tomato culture, characteristic leaf lesions have been produced on pepper plants by atomizer inoculation; and with a culture isolated from bacterial spot on a pepper fruit, typical foliage infection has been secured on tomato.

With a tomato culture, characteristic leaf lesions have likewise been produced on potato plants by atomizer inoculation. These lesions were small, sunken, black dots on the lower surfaces of the leaves.

To summarize, it may be said that tomato foliage infection showed up two to five days after atomizer inoculation, that infection occurred

more abundantly through the lower leaf epidermis, that the young leaves are more susceptible, and that typical infection was obtained with the organism from pepper. Infection of pepper and potato foliage has also been obtained.

FRUIT INOCULATION

On November 21 numerous punctures were made in a green fruit and two ripe fruits with a flamed needle which was frequently dipped in a bouillon culture of the organism. No infection occurred on the ripe fruits. After 11 days dark discolored areas were noted about the punctures in the green fruit and small blackened cores were found about such punctures when the surface of the fruit was sliced off. With a sterile scalpel, portions of this blackened tissue not in contact with the needle channel were removed and crushed in sterile water. In agar plates poured from this inoculum the causal organism was recovered.

On November 20 numerous very shallow needle punctures were made in two green tomatoes which were suspended in moist chambers and sprayed with a bouillon culture. Infection occurred about most of the needle wounds in the shape of submerged, firm, blackened cores 2 to 3 mm. in diameter.

On December 8 five green fruits and one half-ripe fruit were lightly punctured with a needle which was repeatedly dipped in a bouillon culture. Two other green fruits were similarly wounded with a sterile needle. Fourteen days later infection had occurred about most of the punctures on four of the green fruits but not on the fifth which had ripened in the meantime nor on the fruit which was ripening when inoculated. The two controls remained free from infection. A successful reisolation of the organism was made from one of the inoculated fruits.

On February 25 two green fruits were wrapped in cotton which was then saturated with a water suspension of the organism. By the subsequent addition of sterile water the cotton was kept wet four days. This test was later repeated, but no infection of the fruit occurred. On March 25 this test was repeated with two green fruits which were punctured with a sterile needle before applying the cotton and inoculum. At the end of six days infection was evident about many of the punctures, and the lesions were rather shallow and more nearly resembled natural infection (Pl. 24, F).

On March 2 eight small and two large green fruits were punctured with a needle dipped in a water suspension of the organism. All showed infection about the needle wounds.

In April, celluloid cylinders plugged with cotton were suspended about four fruit clusters to act as damp chambers. These fruits were punctured with a sterile needle and sprayed with a water suspension of the organism. At the end of 10 days infection was visible about most of the wounds.

To obtain more minute puncture wounds, small glass tubing was drawn out to very fine capillary points which were reinforced with a block of paraffin, leaving only about 1 mm. of the point projecting. On May 8 eight green fruits were punctured with these glass points and wrapped in absorbent cotton. In four of these cases the cotton was moistened with a water suspension of the organism and in the other four with sterile water for controls. Abundant infection developed about the punctures on the four inoculated fruits and none on the controls. Four green fruits were also punctured with glass points dipped in the inoculum and then covered with moist cotton. All of these likewise showed infection about the punctures. These lesions more closely resembled field infection than those resulting from needle punctures, but even the capillary points apparently produce larger wounds than those through which field infection evidently occurs. None of the uninoculated punctures resulted in the white "bird's-eye" spots which so often accompany bacterial spot in the field. At the same time five fruits which were just beginning to turn yellow and ripen were inoculated by puncturing with the glass points and applying cotton moistened with the inoculum. No infection occurred on these fruits.

A number of ripe and green fruits were picked from the vine, punctured with the glass points, placed in glass moist chambers and sprayed with inoculum. No infection occurred. This would indicate that no infection is likely to occur after the fruits are picked.

In a later series of inoculations of ripening fruits, numerous very small jet-black spots with irregular, lace-like borders were obtained. These lesions were somewhat suggestive of the fruit blemish termed "blotch" in the parlance of market pathology. While reisolation from these lesions was unsuccessful, these results render it unsafe to state that mature fruits are absolutely immune to infection.

Needle prick inoculations in green tomatoes with the organism isolated from pepper bacterial spot have resulted in lesions similar to those produced by the cultures from tomato.

To summarize, it should be noted that: (1) No fruit infection occurred on plants atomized for foliage infection; (2) no infection occurred on unwounded fruits wrapped in cotton saturated with inoculum; (3) infection was obtained by puncturing fruits with a needle or glass point dipped in inoculum; (4) infection was obtained by puncturing the fruits with a glass point or sterile needle and then spraying the surface with inoculum or covering the surface with cotton soaked with inoculum; (5) only rarely, if at all, was infection obtained on mature or ripening fruits, and field observation also indicates that fruit infection occurs only when the fruit is green; (6) the organism from pepper caused typical fruit infection on tomato.

COTYLEDON INOCULATION

Infection on both upper and lower surfaces of the cotyledons was readily obtained by spraying a flat of seedlings with a water suspension of the organism in an atomizer. This infection consisted of small, sunken, silvery or lead-colored, well-scattered spots visible only on one surface of the cotyledon. Secondary infection of cotyledons in flats of seedlings as a result of ordinary sprinkling irrigation was similar in appearance and of common occurrence (Pl. 25, B). Primary cotyledon lesions resulting from seed-borne infection are discussed below.

SEED INOCULATION

On February 12 three small gauze packets (A, B, and C) of tomato seed were dipped in a water suspension of the bacteria from agar slant cultures. Lots A and B were dried at once, and lot C was immersed five minutes in a 10 per cent solution of formalin, rinsed thoroughly, and dried. Lot D consisted of similar seed, not inoculated. Parts of these seed lots were then planted the next day in flats in the greenhouse, and the seedlings were carefully examined at intervals for cotyledon infection.

The results are presented in Table IV.

TABLE IV.—*Seedling infection*

Seed lot.	Treatment.	Number of plants.	Number showing infection after—			Percentage showing infection.
			12 days.	21 days.	27 days.	
A	Inoculated	283	0	24	.....	8½
B	.....do	195	0	26	.....	13
C	Inoculated and treated in formalin	277	0	0	0	0
D	Not inoculated	186	0	0	0	0

Thus, about 10 per cent of the inoculated seed yielded infected seedlings, whereas uninoculated seed and sterilized seed yielded disease-free seedlings. The primary cotyledon lesions were characteristically different from the cotyledon lesions described above. The primary lesions were larger and fewer in number, frequently only one on a cotyledon, more commonly on the dorsal surface, and very often both cotyledons of an affected seedling bore such lesions in corresponding positions. Primary lesions were black, shiny, depressed areas, 2 to 8 mm. in diameter, at first visible on only one surface of the cotyledon (Pl. 25, C). Such lesions were often found associated with the adhering seed coat and frequently occurred at the tip of the cotyledon or at the notch in the margin where the seed coat had remained attached. In cases where such lesions occurred on the midrib, distortion of the cotyledon resulted.

The fact that the seed coat is so often carried up into the air by the cotyledons greatly increases the possibility of cotyledon contamination from the surface flora of the seed. The passage of the organism from the seed coat to the cotyledon should be readily facilitated by the continuous film of water which is often noted on the adhering seed coat.

On March 9, 26 days after inoculation, some of the seeds from lots A and C were planted in sterile sand in glass damp chambers. Seventeen days later, 12 of the 115 seedlings from lot A, or about 10 per cent, showed primary cotyledon lesions, and none of the seedlings from the disinfected seed of lot C showed infection. One hypocotyl lesion was noted.

To summarize the results of these tests, it should be noted that: (1) Inoculated seeds gave rise to diseased seedlings; (2) about 10 per cent of the seedlings showed primary cotyledon lesions; (3) primary lesions were characteristic and seemed to bear a distinct relation to the seed coat; (4) 26 days of drying did not reduce the incidence of primary infection; (5) surface disinfection of inoculated seed prevented seedling infection.

RELATION OF PARASITE TO HOST TISSUE

MODE OF ENTRY

Field observation indicates that fruit infection occurs through an insect puncture. Inoculation tests prove that no infection of fruit occurs unless the epidermis of the fruit is mechanically punctured. With a bacterial disease this condition is to be expected, since there are no natural openings such as stomata in the epidermis of a tomato. The inoculum does not necessarily need to be inserted into the puncture, but when applied to the surface of the fruit the bacteria may enter fresh punctures previously made. Sections through natural lesions sometimes reveal the presence of a puncture wound (Pl. 27, A). A species of stink bug has been observed puncturing green tomato fruits in the field, and infection might readily occur through wounds caused by this insect.

Leaf infection apparently occurs through stomata. The abundance of the lesions resulting from inoculation of the lower leaf surfaces as compared with the scarcity of lesions resulting from inoculating the upper surfaces has been previously noted. To illustrate this relation a further analysis of one of the greenhouse inoculation trials is presented in Table V. Each pair of plants received similar treatment in all respects except as noted. Atomizer inoculation was used. To facilitate inoculation of the lower epidermis of the leaves, the small potted plants were suspended upside down in a ring stand.

TABLE V.—*Relative susceptibility of upper and lower epidermis*

Plant.	Inoculation.	Total number of leaflets.	Leaflets showing infection.			
			Total number.	Percentage.	More than 10 lesions.	
					Number.	Percentage.
A 41	Lower epidermis	32	18	56	10	31
A 42	Upper epidermis	36	10	28	1	3
B 44	Lower epidermis	43	30	69	22	51
B 45	Upper epidermis	40	23	57	10	25
C 44	Lower epidermis	32	7	22	5	16
C 45	Upper epidermis	33	1	3	0	0
D 44	Lower epidermis	39	29	74	27	69
D 45	Upper epidermis	40	6	15	1	3
E 41	Lower epidermis	37	18	49	14	37
E 42	Upper epidermis	27	6	22	6	22
Averages.	Lower epidermis	.....	.....	54	.....	41
	Upper epidermis	.....	.....	25	.....	10

Thus, it is seen that the average percentage of infected leaflets on plants atomized from below is 54 as compared with 25 on plants atomized from above, and that the difference is still more striking when the percentages of heavily infected leaves are compared, in which case the figures are 41 and 10, respectively.

This decidedly higher incidence of infection through the lower epidermis suggested some connection with the distribution of stomata. To throw further light on this point, portions of the epidermis from the upper and lower surfaces of several leaves were removed and mounted so that stomatal counts could be made under the microscope. The results are presented in Table VI.

TABLE VI.—*Number of stomata per square millimeter of epidermis*

Source of epidermis.	Upper epidermis.	Lower epidermis.
Young plant, young leaf	50	117
Young plant, old leaf	5	98
Do	0	131
Old plant, young leaf	3	246
Do	11	306
Cotyledon	41	93
Do	83	61

Thus, it is readily seen that only on cotyledons and young leaves on young plants are there numerous stomata in the upper epidermis, whereas the stomata are abundant in the lower epidermis of leaves of all ages. Thus the distribution of stomata is correlated with the incidence of infection; and on the basis of stomatal entry, the greater abundance of lesions resulting from inoculation of the lower epidermis and the greater susceptibility of young leaves to inoculation of the upper epidermis is explained. The more or less equal distribution of the stomata on the cotyledons also explains the fact that cotyledon lesions occur with equal frequency on either surface. Petioles were found to have about 34 stomata per square millimeter.

Sections for microscopic study were cut from infected cotyledons fixed nine days after inoculation. These were stained in carbol fuchsin. Plate 28, C, is a photomicrograph showing a mass of the bacteria in a substomatal chamber directly under a stoma in the upper epidermis of the cotyledon. This affords evidence of stomatal entry.

RELATION OF HYDROGEN-ION CONCENTRATION TO INFECTION

An approximate determination of the hydrogen-ion concentration of different parts of greenhouse tomato plants was made by the colorimetric method described by Clark and Lubs (*1*). The plant juice was extracted by crushing the leaves or fruit in a mortar. This juice was diluted 1 to 5 or 1 to 20 with distilled water of a tested P_H value and titrated by the addition of the indicators brom thymol blue, brom cresol purple, and methyl red.

Seedlings and leaves yielded a P_H value between 6.3 and 6.5. Green fruits showed P_H values between 5 and 5.4, and in all cases the pericarp was slightly less acid than the seed pulp. Ripening and mature fruits showed P_H values of about 4.6. According to Clark and Lubs (*1*, *p. 221*), Patten and Mains found the P_H value of ripe tomatoes to be 4.2, and other workers have given it as 4. Thus it is evident the hydrogen-ion concentration in fruits is higher than in the foliage and is much higher in ripe fruit than in green fruit.

There is an interesting correlation between these P_H values, the hydrogen-ion tolerance of the bacterial spot organism in culture, and the observed susceptibility of the host parts to infection. In the colorimetric determination of the hydrogen-ion tolerance in culture, the upper limit of tolerance was a P_H value of 5.3 for ordinary inoculation, although reinoculation resulted in raising this limit to 5. The seedlings and leaves are, therefore, well within the hydrogen-ion tolerance of the organism, the green fruit or at least the pericarp is just within the limit of tolerance, while ripening and mature fruit has a much higher hydrogen-ion concentration than the organism will endure in culture. Seedlings, leaves, and green fruit are very susceptible to the disease, whereas, as a

rule, inoculation of ripe fruit has been unsuccessful. It seems plausible that the increase in hydrogen-ion concentration as the fruit matures may increase its resistance to infection.

PATHOLOGICAL ANATOMY

Leaf lesions were fixed in alcohol, embedded in paraffin, sectioned, and stained with Ziehl's carbol fuchsin. The intercellular spaces of the palisade and mesophyll tissue are seen to be packed with bacteria (Pl. 28, C). Apparently as a result of collapse of the host cells, these cavities become greatly enlarged and the host cells are more or less displaced by the masses of bacteria. The epidermis may finally become puffed outward as a result of the pressure produced. The subsequent sunken character of the lesion is attributed to the drying out of this mass of bacteria and killed host tissue. The silvery appearance of sunken cotyledon lesions is attributed to the presence of air under the epidermis after the diseased tissue has dried and shrunken.

Infected leaf areas were cleared in acetic acid and alcohol, or in chloral hydrate, and stained with carbol fuchsin. In surface view the lesions are clearly visible each as a deeply stained, lacelike pattern (Pl. 28, A). This indicates that the bacteria are in the intercellular spaces. The margin is composed of irregular extensions due to advance invasion of the intercellular spaces. The veins offer no obstacle to the progress of the invasion.

Hand sections of fruit lesions show an abundance of bacteria in the intercellular spaces. Fruit lesions were fixed and stained with the triple stain or with carbol fuchsin. Very young lesions may be distinctly sunken on account of the collapse of the outer mesocarp cells. Older lesions are seen to be distinctly elevated at the center, apparently as a result of hypertrophy and hyperplasia of the underlying mesocarp cells (Pl. 27, B, C). Such lesions often show a sunken peripheral zone, due to the collapse of the host cells. A layer of cells with suberized walls is formed directly underneath the necrotic tissue of the center of the lesion (Pl. 27, B). Usually the epidermis has sloughed off from this disintegrated tissue which is composed of collapsed host cells and bacteria. Large cavities packed with bacteria are visible here and there. In Plate 27, A, the large cavity may represent the original wound through which infection occurred.

Apparently the cork layer is not always an effective barrier to the invasion, since lesions often are deep and craterlike. Tangential invasion of the small-celled mesocarp tissue seems to be more active than radial penetration of the large-celled mesocarp tissue.

OVERWINTERING AND DISSEMINATION

Whether or not the causal organism of bacterial spot overwinters in the soil has not been proved as yet. It has been found, however, that the organism lives over winter on tomato seed and that such seed gives rise to infected seedlings.

Laboratory tests have proved that the organism will endure long periods of desiccation upon tomato seed. In one test not yet completed the organisms have endured over 11 months of such desiccation.

Tomato seed immersed in a water suspension of the bacteria, dried, and then planted in sterilized soil gave rise to numerous infected seedlings. The primary cotyledon lesions were very characteristic and fairly easily differentiated from secondary infection.

Commercial seed was then tested in a similar manner. A number of lots of commercial seed from the fruit in a large field known to have been severely diseased with the bacterial spot were tested by planting in flats of sterilized soil in the greenhouse during April and May of 1920. This seed had been machine-cleaned from the tomato pulp in a canning factory during the 1919 harvest, and in this process of seed separation and cleaning there was ample opportunity for contamination of the seed with the bacteria from fruit lesions. The results of two series of such tests are presented in Table VII.

TABLE VII.—*Test of commercial seed in sterilized soil*

Seed lot.[1]	Number of plants.	Number of plants with infection.	
		Primary.	Secondary.
8 a	309	0	0
8 b	490	4	18
9 a	318	5	4
9 b	364	6	6
10 a	271	1	2
10 b	509	6	4
19 a	336	0	1
19 b	557	2	6
21 a	334	0	0
21 b	490	10	36
22 a	324	6	17
22 b	500	8	40
23 a	299	4	3
23 b	404	4	1
25 a	281	4	1
25 b	374	6	0
26 a	330	10	26
26 b	397	5	0
Total	6,887	81	165

[1] Part of seed lot designated as "a" planted Apr. 14; results taken 31 days later. Part designated as "b" planted Apr. 24; results taken 26 days later.

Thus it is apparent that 81 out of 6,887 seeds, or about 1 per cent, gave rise to diseased seedlings. This proves that the organism occurs on commercial seed and lives over winter thereon and that such seed yields diseased seedlings. As a means of dissemination about 1 seed out of every 100 should be expected to give rise to a center of infection, and with such seed the disease will be carried far and wide. Field observations indicate such to be the case. The disease was found in fields in Georgia planted with seed sent from the North. These fields were not in tomatoes before, and it seems quite likely that the disease was introduced with the seed. Furthermore, the disease appeared in plant beds in clean soil in southern Indiana planted with seed obtained from a diseased field the previous season. This seed was hand-cleaned by cutting the fruits in two and rubbing the halves over a screen. This process undoubtedly facilitated seed contamination from fruit lesions. It is evident, therefore, that the disease may be transported long distances and introduced into new fields and localities by contaminated seed.

Judging from the outbreak of the disease among the seedlings in the Georgia fields and in the plant b ds at Campbellsburg, Ind., it seems quite likely that considerable spread of infection may occur in the plant beds before the plants are transplanted to the field and that the disease may be carried long distances and introduced into new fields with diseased transplants. The disease has been found epidemic early in the season in an Indiana field planted with Georgia plants.

Local spread of infection has not been carefully studied. The occurrence of secondary infection in the greenhouse flats indicates that infection is spread by the sprinkling process. Little or no secondary infection occurred on plants which were not sprinkled. In the field infection is undoubtedly spread by the splashing of rain, by wind-blown rain, and by surface drainage water.

The relation of insects to fruit infection in the field still remains to be worked out. That fruit infection occurs through insect punctures seems almost unquestionable. Whether or not the insect inoculates the fruit at the same time the wound is made has not been determined. Greenhouse tests indicate that infection may occur through fresh punctures already made, as well as in punctures made with an inoculated instrument.

In summary it may be said that: (1) The organism lives over winter on the surface of the seed; (2) among seedlings grown from commercial seed, about 1 in every 100 may bear primary cotyledon lesions and serve as a center of infection; (3) the disease is disseminated by contaminated seed and by diseased transplants.

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CONTROL

Since the causal organism of bacterial spot is carried with the seed, seed disinfection at once suggests itself as a control method.

Inasmuch as the fruit lesions are superficial, there is no probability that the bacteria are inside the seed, and all evidence points toward their being carried on the exterior of the seed. The fruit lesions contain millions of the bacteria, and the processes of extracting the seed afford ample opportunity for surface contamination of the seed with the bacteria from these lesions. The surface of a tomato seed is covered with long cellulose hairs which increase immensely the surface for lodgment of the organisms and prevent their removal by washing. Laboratory tests have proved that the organisms will endure long periods of desiccation on seed. Surface disinfection of the seed should eliminate the disease-producing bacteria.

A number of tests were made to determine what method of disinfection might be safely applied to tomato seed. Small quantities of tomato seed were tied up in gauze bags. Before being placed in the disinfecting solution each bag was immersed about a minute in 50 per cent alcohol to remove air bubbles from the hairy seed coats. After disinfection the seed samples were dried, and portions were submitted to the local Federal seed laboratory for germination tests. Part of the samples were tested in soil. The results are presented in Table VIII.

TABLE VIII.—*Effect of seed disinfection on vitality of seed*

Seed sample No.	Treatment.	Percentage of germination in official test.	Percentage of germination in soil flats.
1	$HgCl_2$ 1:1,000, 5 minutes	87.5	72
2	$HgCl_2$ 1:2,000, 5 minutes	94.5	80
3	Formaldehyde 1 per cent, 5 minutes	96.5	.....
4	Formaldehyde 2 per cent, 5 minutes	96.5	92
9	Control, untreated	95	96
5	$HgCl_2$ 1:1,000, 5 minutes	62.5	.....
6	$HgCl_2$ 1:2,000, 5 minutes	85	.....
7	Formaldehyde 1 per cent, 5 minutes	87	.....
8	Formaldehyde 2 per cent, 5 minutes	87	.....
10	Control, untreated	86	.....
13	$HgCl_2$ 1:3,000, 5 minutes	88	86
14	$HgCl_2$ 1:4,000, 5 minutes	90	79
15	$HgCl_2$ 1:10,000, 5 minutes	87.5	.....
16	Formaldehyde 2.5 per cent, 10 minutes	86.5	81
17	Formaldehyde 2.5 per cent, 5 minutes at 50° C.	74.5	.....
18	Formaldehyde 5 per cent, 5 minutes	89.5	81
19	Formaldehyde 10 per cent, 5 minutes	89.5	88
20	Hot water, 55° C., 10 minutes	68	49
21	Control, untreated	85	80 86

From these figures it is evident that treatment in mercuric chlorid 1 to 1,000, in hot water, and in hot formaldehyde is injurious to germination. None of the cold formaldehyde treatments resulted in

injury, and in the soil germination tests some of these treatments resulted in increased vigor as compared with the untreated controls.

To test the effectiveness of these treatments, some of the seed, after treatment, was rinsed in sterile water rather than tap water and dried. Seeds were then planted in agar poured plates. Perfect sterilization was obtained only with the mercuric-chlorid treatments, and of these the weaker strengths seemed to be as effective as the more concentrated. The hot water gave only 24 per cent sterile seed, the 10 per cent formaldehyde gave 36 per cent, and the 5 per cent formaldehyde gave only 4 per cent. The hot formaldehyde gave only 16 per cent sterile seed. Thus the mercuric-chlorid treatments are effective and the formaldehyde treatments are not thoroughly effective against the ordinary surface flora of the seed.

However, tests with the causal organism of bacterial spot in suspension had shown it to be killed by 5 minutes' exposure to 2 per cent, 5 per cent, and 10 per cent formaldehyde and to mercuric chlorid 1 to 2,000 and 1 to 4,000. To test the effectiveness of the formaldehyde and mercuric-chlorid treatments on inoculated seed, a quantity of seed was sterilized in water in the autoclave, dipped in a suspension of the organisms, and dried in sterile Petri dishes. Then some of this seed was tied up in gauze bags and immersed in the disinfecting solution 5 minutes, rinsed in sterile water, and dried in Petri dishes. These seeds were then tested by planting in agar poured plates, with results as presented in Table IX.

TABLE IX.—*Effectiveness of seed disinfection (inoculated seed)*

Treatment (5 minutes).	Number of seeds tested.	Number showing bacterial growth.	Percentage of effectiveness.
Control, untreated	50	49	.....
2 per cent formaldehyde	60	8	87
5 per cent formaldehyde	60	7	88
10 per cent formaldehyde	60	3	95
5 per cent formaldehyde after immersion in 40 per cent alcohol	80	7	91
5 per cent formaldehyde after immersion in 10 per cent alcohol	80	5	94
Control, untreated	100	92	.....
$HgCl_2$, 1: 2,000	100	0	100
$HgCl_2$, 1: 3,000	100	0	100
$HgCl_2$, 1: 4,000	100	0	100

Thus, it is seen that the formaldehyde treatments do not perfectly sterilize all the inoculated seed even where perfect wetting is insured by a preliminary immersion in alcohol. On the other hand, perfect sterilization was obtained with mercuric chlorid 1 to 2,000, 1 to 3,000, and 1 to 4,000.

To determine more accurately the effect of these mercuric-chlorid treatments upon the viability of the seed, germination tests similar to those recorded in Table VIII were repeated, using soil flats in the greenhouse. The results are presented in Table X.

TABLE X.—*Effect of mercuric chlorid upon germination*

Treatment.	Number of seeds.	Percentage of germination after—			Remarks.
		5 days.	7 days.	13 days.	
$HgCl_2$, 1: 2,000	100	54	87	97	No retardation.
$HgCl_2$, 1: 3,000	100	47	67	93	Do.
$HgCl_2$, 1: 4,000	100	12	58	85	Do.
Control	100	.....	87	93	
$HgCl_2$, 1: 2,000	200	.....	91. 5	.....	Do.
$HgCl_2$, 1: 3,000	200	.....	96	.....	Do.
$HgCl_2$, 1: 4,000	200	.....	90	.....	Do.
Control	200	.....	87	.....	

Thus, the weaker strengths of mercuric chlorid have no deleterious effect upon the germinability of the seed nor the vigor of the seedlings. Pending subsequent tests, disinfection of tomato seed in 1 to 3,000 mercuric chlorid for five minutes followed by thorough rinsing in running water is, therefore, recommended as a control for tomato bacterial spot.

To summarize, it may be pointed out that: (1) Seed treatment in hot water (55° C. for 10 minutes), in hot formaldehyde (2.5 per cent at 50° for 5 minutes), and mercuric chlorid 1 to 1,000 for 5 minutes was injurious to the seed; (2) 5 per cent and 10 per cent formaldehyde exerted no injurious effects but were not reliably effective; (3) mercuric chlorid 1 to 2,000, 1 to 3,000, and 1 to 4,000 gave perfect sterilization and exerted no injurious effects.

SUMMARY

Bacterial spot of tomato is a typical spot disease of the fruits, stems, and foliage.

Practically all varieties of tomatoes are susceptible. Peppers and potatoes are also susceptible.

The disease as it occurs on tomato fruit was first reported from Tennessee, Illinois, and Michigan. It was called "canker." It has a wide geographic range.

The worst damage is due to the fruit lesions. The disease is also destructive among seedlings and occasionally as a foliage trouble in the field.

The fruit lesions are small, black, scablike spots, usually superficial, sometimes crateriform. Leaf lesions are at first translucent, later black and greasy with translucent margins, and are not usually limited by the veins.

The causal organism is a monoflagellate bacterium which is described herein as *Bacterium exitiosum*, n. sp. It grows readily on a variety of culture media, producing yellow, translucent colonies. It produces no acid or gas with carbohydrates and is highly sensitive to sunlight and very resistant to desiccation. In culture it will not tolerate a higher true acidity than P_H 5.

Foliage infection is stomatal and is readily obtained by atomizer inoculation. Fruit infection occurs only through puncture wounds. The invasion is intercellular at first. Inoculation of mature fruit is usually unsuccessful and is attributed to the fact that the hydrogen-ion concentration in mature fruit (P_H 4.6 to 4) is higher than that tolerated in culture. However, green fruit and foliage yield P_H values within the range of tolerance of the organism in culture.

The organism overwinters on the surface of tomato seed and is thus disseminated. Commercial seed from fields known to be diseased has yielded about 1 per cent of diseased seedlings. The disease is also disseminated with diseased transplants.

As a control measure disinfection of tomato seed in mercuric chlorid 1 to 3,000 for 5 minutes, followed by thorough washing, is tentatively recommended as safe and effective.

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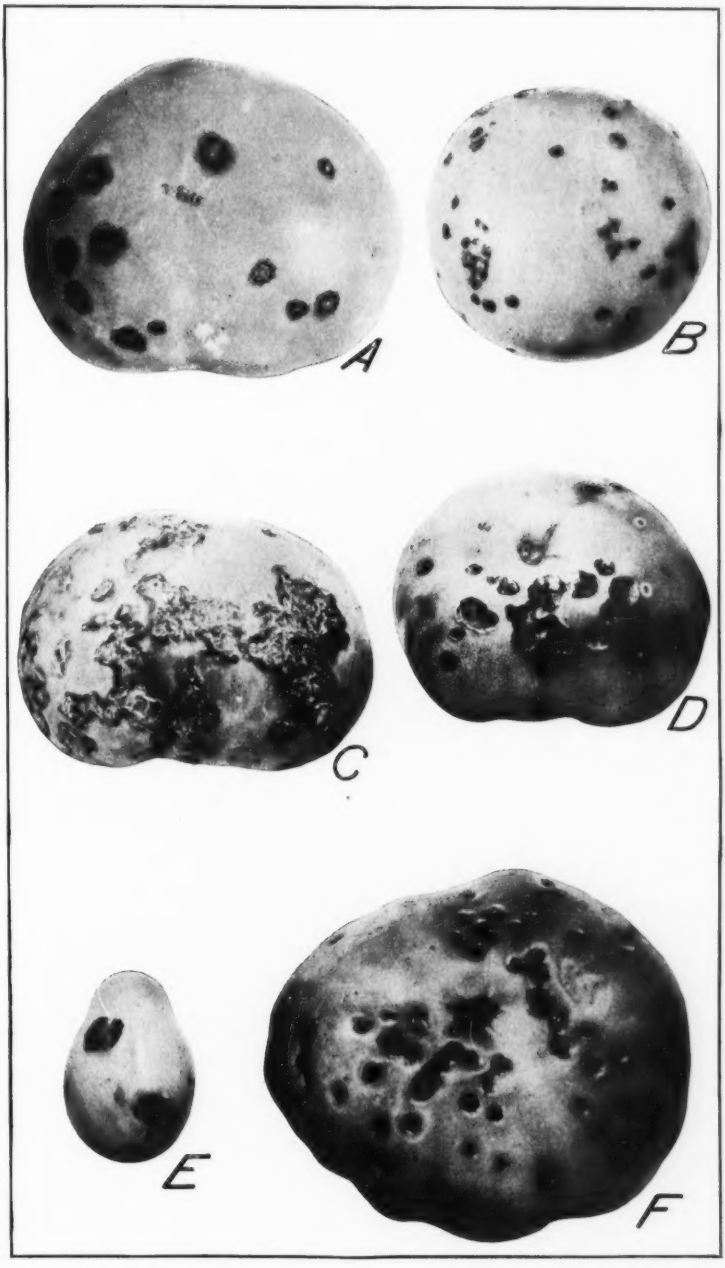
PLATE 24

Bacterial spot of tomato:

A.—Fruit lesions with scablike centers and water-soaked borders.
B.—Very young fruit lesions in the black-scab stage.
C.—Severe blemishing of fruit due to the coalescence of lesions.
D.—Pitlike or crateriform fruit lesions. Two of the white, circular, "bird's-eye" spots are also present.
E.—Large lesions on yellow-pear tomato.
F.—Lesions resulting from needle-prick inoculation of fruit in greenhouse.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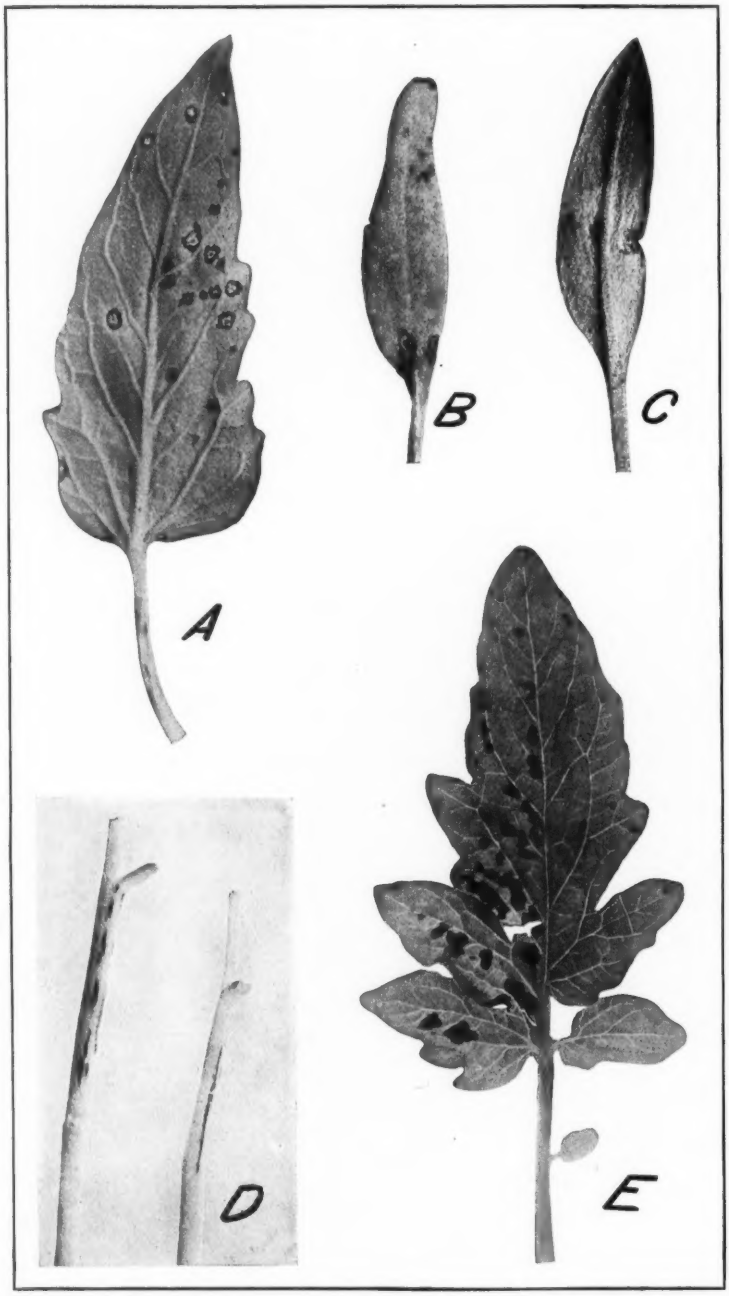
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PLATE 25

Bacterial spot of tomato:

A.—Leaf lesions resulting from atomizer inoculation in greenhouse.
B.—Cotyledon showing primary lesions near base and secondary lesions near tip.
C.—Cotyledon showing typical sunken primary lesions on lower surface.
D.—Black, linear, petiole lesions.
E.—Leaf lesions resulting from natural infection in the field. These lesions are black and greasy, with a narrow translucent margin.

PLATE 26

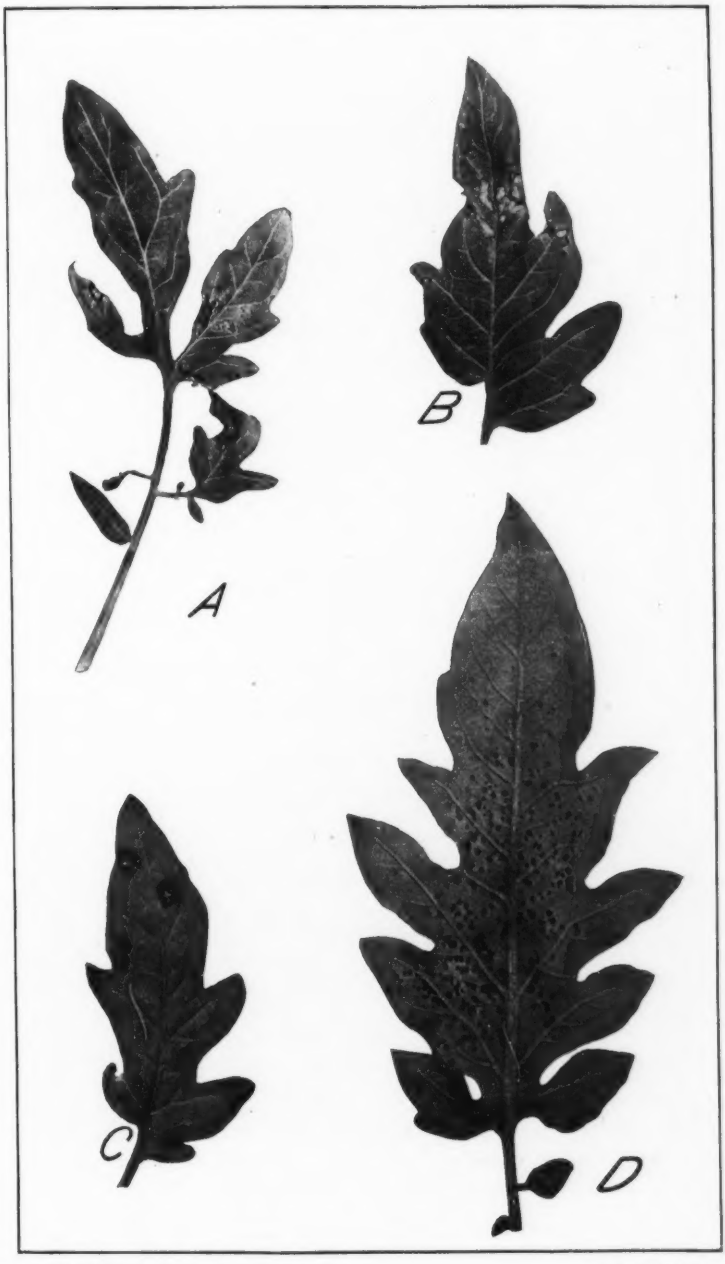
Bacterial spot of tomato:

A.—Distorting effect of lesions upon a young leaf.

B.—Distortion of leaflet as a result of very early infection from atomizer inoculation in greenhouse.

C.—Old lesions resulting from atomizer inoculation of a young leaflet.

D.—Type of infection secured by atomizer inoculation of an old leaf. Thick-set, small, circular, sunken lesions on lower surface.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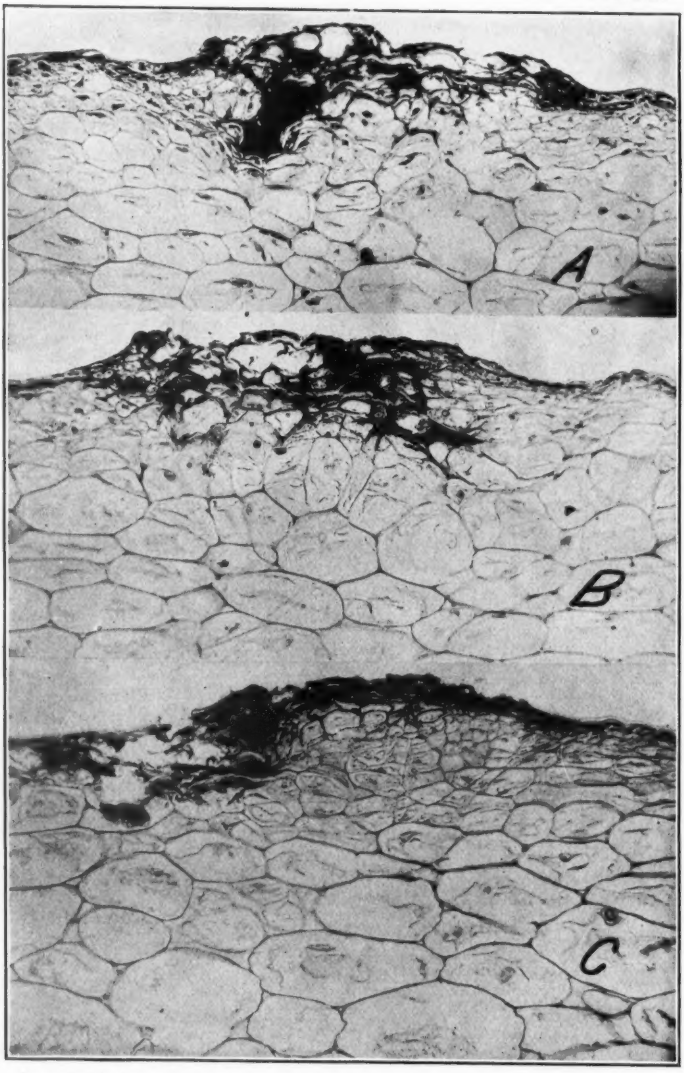
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PLATE 27

Bacterial spot of tomato:

A.—Cross section of young fruit lesion resulting from natural infection. What appears to be a puncture wound is clearly visible. Photomicrograph, × 90.

B.—Cross section of young fruit lesion, showing hypertrophy of underlying mesocarp cells and formation of a cork layer under the necrotic tissue. Photomicrograph, × 90.

C.—Cross section of a fruit lesion, showing hyperplasia of mesocarp cells and formation of a cork layer at one side of the lesion. Photomicrograph, × 90.

PLATE 28

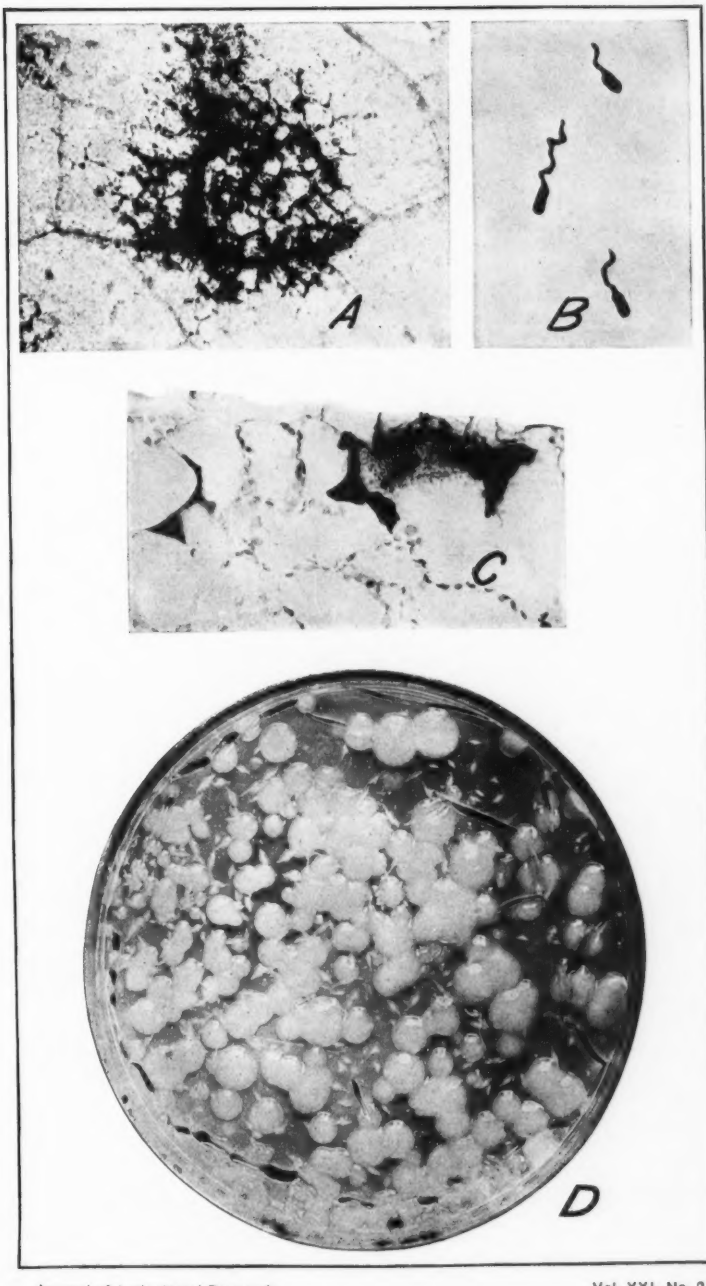
Bacterial spot of tomato:

A.—Surface view of a leaf lesion stained to show distribution of bacteria. The invasion is plainly intercellular and the margin not delimited by veins. Photomicrograph, × 66.

B.—Causal organism stained to show flagella. Photomicrograph, × 2,000.

C.—Cross section of young cotyledon lesion, showing a mass of bacteria under a stoma and intercellular spaces packed with bacteria. Photomicrograph, × 300.

D.—Poured plate culture of causal organism on potato agar.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CARBON TETRACHLORID FOR THE REMOVAL OF PARASITIC WORMS, ESPECIALLY HOOKWORMS

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Experiments carried on in the Zoological Division of the Bureau of Animal Industry indicate that carbon tetrachlorid (CCl_4) is a very promising drug for the treatment of hookworm infestation in dogs. The experimental results are therefore presented in this paper, primarily to bring the drug to the attention of veterinarians for use in cases of canine ancylostomiasis, and secondarily to make the results public for consideration in connection with ancylostomiasis in man and in domesticated animals other than the dog. The paper includes a consideration of the efficacy of the drug, a study of its effect on dogs ante mortem and post mortem, and a résumé of some of the more readily available literature on carbon tetrachlorid, especially those papers dealing with the therapeutics of this drug.

MEDICAL LITERATURE

The United States Dispensatory,[1] nineteenth edition, issued in 1907, states in regard to carbon tetrachlorid that it was discovered by Regnault in 1839. Carbon tetrachlorid may be made from carbon bisulphid (CS_2) or from chloroform ($CHCl_3$) by chlorination. It is a solvent of fats, alkaloids, rubber, sulphur, etc., and is used as a fire extinguisher.

Tuson (*21*) in 1843 reported carbon tetrachlorid as of value when applied externally to cancers in a lotion composed of 1 dram of carbon tetrachlorid to 1 pint of water, and given internally in doses of 1 to 3 drops in water three times a day. The cancer ultimately sloughed away, leaving healthy tissue. Carbon tetrachlorid was of value in other similar cases and also in nervous irritability and allied conditions.

Ure (*22*) in 1843, following Tuson's directions, used carbon tetrachlorid by application to a cancer of the tongue, to an erosive ulceration of the throat, and to a cancer of the leg without benefit in any case.

Blanch (*2*) in 1843 reported that 4 drops of carbon tetrachlorid three times a day gave good results in gout. In a patient with severe neuralgic pain and with pronounced aversion to drugs, 1 tablespoonful of a preparation containing 6 ounces of water and 1 dram of carbon tetrachlorid gave relief following the first dose and complete relief after the third or fourth. In a case of diarrhea, procidentia uteri, prolapsus ani, hysterical and fainting fits, and insomnia, carbon tetrachlorid by mouth relieved the symptoms and enabled the patient to sleep.

[1] WOOD, Geo. B., and BACHE, Franklin. THE DISPENSATORY OF THE UNITED STATES OF AMERICA. Ed. 19. . . lxii, 1,947 p. Philadelphia, [1907].

Sansom (*16*)[2] in 1865 reported the findings of himself and Harley thus: Carbon tetrachlorid—

causes, first, muscular movement and excitation of circulation. Secondly, arterial contraction and anesthesia. Thirdly, when long continued, arrest of respiration. Fourthly, arrest of circulation. Tendency to cause muscular rigidity.

According to the Dispensatory, Simpson found that the vapors would relieve some cases of conjunctivitis, ulceration of the cornea, photophobia, etc., but in Simpson's paper (*19*) published in 1865 the vapors referred to are those of chloroform. Subcutaneous injections of 10 to 20 minims relieved pain in the walls of the chest and abdomen, without subsequent nausea. Simpson concluded that it depressed the heart much more than chloroform did; and, apparently as a result of this belief on the part of Simpson and other investigators, the drug has not come into general use and has been almost entirely neglected in medicine for half a century.

An examination of Simpson's paper discloses the following observations:

Its primary effects are very analogous to those of chloroform, but it takes a longer time to produce the same degree of anesthesia, and generally a longer time to recover from it. Some experiments with it upon mice and rabbits have shown this. . . . But the depressing influence of chlorocarbon [carbon tetrachlorid] upon the heart is greater than that of chloroform; and consequently I believe it to be far more dangerous to employ as a general anesthetic agent. In a case of midwifery in which it was exhibited . . . for above an hour, with the usual anesthetic effects, the pulse latterly became extremely feeble, and weak. In another case in which it was exhibited . . . her pulse continued steady and firm, although she is the subject of valvular disease of the heart. The surgical operations . . . the closure of a vesico-vaginal fistula, the division of the cervix uteri, the enlargement of the orifice of the vagina, and the application of potassa fusa to a large flat nævus upon the chest of a young infant—in all of these cases it answered quite well for an anesthetic. The child did not waken up for more than an hour and a half. . . . Its pulse was rapid and weak during the greatest degree of anesthetic sleep. One of the mice exposed to its influence, and which was removed from the tumbler, where the experiment upon it was made, as soon as the animal fell over, breathed imperfectly for some time after being laid on the table and then died.

Without caring to pass on the question as to whether carbon tetrachlorid is a more powerful heart depressant than chloroform, it may still be said that the foregoing data hardly warrant such a conclusion. The exhibition of chloroform for over an hour may also cause a feeble pulse, and the exhibition of the drug to a patient with valvular disease of the heart, with persistent, firm pulse, speaks well for the action of carbon tetrachlorid. The experiment with the mouse could be duplicated by the rapid administration of a concentrated chloroform mixture. Naturally, one must proceed with caution in applying in veterinary practice a drug of which the toxic properties are not well known, and the same is even more true of the administration of the drug to man.

Apparently the most comprehensive study of the administration of this drug to man is that of Smith (*20*) in 1867, who has reported the

[2] Reference is made by number (italic) to "Literature cited," p. 174-175.

administration of the drug on 52 occasions, almost always with satisfactory results. He concludes:

> The observations thus briefly reported may offer sufficient ground to justify the belief that the tetrachlorid of carbon, carefully administered, will be found useful in removing pain, especially headache, dysmenorrheal distress, tic douloureux, toothache, etc.; that it will be a valuable and safe means of mitigating the sufferings of labor without, apparently, hindering the natural efforts; in some cases, of inducing quiet sleep, and of removing for a time the effects of exhaustion of the nervous system. Its vapor, acting locally, seems to have been beneficial in alleviating the distressing irritation of "hay fever" in the few cases in which it has been tried; and when used per vaginum, in exerting a soothing influence and relieving pain. Like all anesthetics, if recklessly used it might destroy life; yet, carefully managed, it may with impunity be employed to induce complete anesthesia. In the majority of cases there has been no nausea nor sickness following its use . . . whilst it has been often observed that the relief from pain obtained by it continued after its immediate anesthetic effects have disappeared. . . .

Sansom (*17*, *18*) reported in 1867 on the conditions in which the drug has been found beneficial, and reaches the following conclusions:

> In cases of natural labor, the tetrachloride has been employed . . . and in none of these cases . . . has it manifested any unfavorable effect; and it has greatly relieved, if it has not altogether abolished the suffering . . . It increased muscular power, and certainly in no case did it suspend the efforts of labor. In the performance of surgical operations a state of narcotism is necessary; and it would appear that the prolonged employment of the tetrachloride in such cases is undesirable and likely to be injurious. So far as the earlier stages are concerned, the action of the tetrachloride is beneficial, as it is stimulant, anodyne and hypnotic, and produces no unpleasant effect; but its ponderous vapor, its insufficient volatility, and the consequent difficulty of its elimination from the system, are sufficient reason against its employment in anything like large doses.

A consideration of the reports of Simpson, Smith, and Sansom shows the most favorable results, except as regards the idea that carbon tetrachlorid is more depressant to the heart than chloroform and that its lesser volatility and supposed difficulty of elimination are objections to its use in large doses.

Andrews (*1*) in 1867 reported one test of it as an anesthetic in resection of the hip joint in a patient who was very weak and anemic. Before anesthesia was complete there was a sudden increase in pulse rate and pain in the vicinity of the heart, followed by cessation of heart beat and respiration. The patient was revived by artificial respiration, and the operation was completed under ether. Subsequently the patient died of exhaustion.

Nunneley (*15*) in 1867 tested carbon tetrachlorid on cats and rabbits and on himself. He concluded that it was not a satisfactory and safe anesthetic but found it of value in his own case in bronchial catarrh, the condition clearing up after one inhalation.

Morel (*14*) in 1877, on tests of carbon tetrachlorid, concluded that it was superior to chloroform, though not entirely controllable. It gives

rise to three phases in its effects when inhaled: First, excitation; second, insensibility; third, collapse. The first phase resembles that of ether.

Laffont (*12*) in 1877 notes Morel's results, but after testing the drug on dogs, cats, and frogs is unable to concur in Morel's conclusions. He finds that it acts first on the brain and then on the spinal cord, producing reflexes indicated by active movements. The action of the heart and lungs ceases when the medulla oblongata is affected. In the first phase, that of excitation, there are tonic and clonic movements of a constant character, the heart beat is disordered and augmented, and the arterial pressure rises and then falls, the pupil of the eye being constantly dilated. In the second phase, that of insensibility, the heart beat is very fast, the pulse feeble and the arterial pressure low, the pupil reaching its maximum dilation. In the third phase, that of collapse, the pulse is very feeble, the heart beat slows, and finally respiration and heart beat cease; animals are rapidly revived by artificial respiration. The pupil may contract in the third phase. Carbon tetrachlorid does not affect the oxygen content of the blood. The safety factor in the administration of carbon tetrachlorid may be increased by the preliminary administration of morphine; this eliminates the spasmodic movements. The initial contracted pupil due to morphine gives place to a dilated pupil following the administration of the carbon tetrachlorid. Laffont notes that it requires 12 to 15 gm. to anesthetize dogs weighing 15 to 35 kgm. He does not regard this drug as superior to chloroform.

In the "London Letter" in the Journal of the American Medical Association (*5*) for July 31, 1909, a case is reported where a woman 29 years old, a subject of status lymphaticus, collapsed while being shampooed with carbon tetrachlorid and never recovered. In 30,000 cases where this form of shampoo had been used this was the first fatality, and there had previously been no untoward results other than one or two cases of fainting.

Mac-Auliffe (*13*) in 1916 reported carbon tetrachlorid as a safe and effective substance for use in war in cleansing the margins of wounds.

The British Medical Journal (*4*) for September 25, 1920, has a report of carbon-tetrachlorid poisoning in a girl employed in painting golf-ball molds with a paint containing this substance. The symptoms were vomiting after every meal, headache, and anemia. It is noted by the editor that frequent inhalations of carbon tetrachlorid cause dyspepsia of a toxic type, anemia, and ultimately a toxic jaundice. The occurrence of jaundice is of interest as indicating that carbon tetrachlorid, like chloroform, may have injurious effects on the liver.

Mr. F. C. Bishopp, of the Bureau of Entomology, United States Department of Agriculture, writes under date of December 27, 1920, that carbon tetrachlorid irritates wounds and retards healing and that

two stockmen think that they killed two calves and one steer by its use in cases of screw-worm infestation.

The findings of the various writers cited above are conflicting. Tuson, Blanch, Smith, and Morel report good results; Ure, Andrews, and Laffont report unsatisfactory results; Simpson, Sansom, and Nunneley report good results with distinct reservations as to the danger present in the use of this drug. The last attitude is perhaps the best, as well as the most conservative. The evidence indicates that when inhaled the drug gives rise to an initial excitation comparable to that produced by ether, and that subsequently it acts as a heart depressant comparable to chloroform. Knowing as we do that adequate amounts of chloroform by inhalation or by mouth may give rise to acute yellow necrosis of the liver, it would be expected that carbon tetrachlorid, with a formula very similar to chloroform, would produce somewhat similar effects. Owing to the small amount of work on this drug, the evidence on this point is somewhat meager, but apparently carbon tetrachlorid does cause some injury to the liver. The statement in the British Medical Journal of September 25, 1920, already noted, that frequent inhalations may ultimately give rise to toxic jaundice is such evidence. Additional evidence is furnished me by Dr. Ch. Wardell Stiles, to whose attention these anthelmintic findings have been brought. He states that experiments at the Hygienic Laboratory show that carbon tetrachlorid does cause some injury to the liver. In this connection more study is evidently necessary. The acute yellow necrosis due to chloroform poisoning is sometimes fatal, but when this is not the case there is a complete repair and recovery in the course of two weeks. Apparently the administration of sodium bicarbonate is of value in combating the acidosis present in such cases. The capacity of the liver for disposing of poisons and for regeneration makes it the organ of choice for anthelmintic insult.

On the available evidence carbon tetrachlorid deserves further study in connection with its administration in labor. That it relieves pain without diminishing the uterine contractions would indicate that it is superior to chloroform and should be restudied in connection with present-day interest in the production of the so-called "twilight sleep." It seems to warrant further study in connection with its employment in hay fever, hysterical headache, and the other conditions in which it gave such good results.

The foregoing facts are given in some detail for the reason that they cover fairly well what is known about this drug, so far as literature may be readily traced, and because, in suggesting the employment in a new rôle of a substance which is not well known, it seems advisable to state what is known about its physiological action in connection with the first report of its anthelmintic action.

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ENTOMOLOGICAL LITERATURE

Another body of literature on carbon tetrachlorid which may be only briefly mentioned here is that covering its use as an insecticide. Smith (*20*), in 1867, noted that chloroform dropped on the head of test insects killed them, whereas carbon tetrachlorid had only a transient, bad effect on them, the insects recovering. Britton in 1908 used it for scale insects on nursery trees. Morse in 1910 used it against insects in grain and in natural-history specimens and concluded that it required twice as much of it as of carbon bisulphid. Pettit, Yothers, and Shafer tested it in 1910, and the report was published by Shafer in 1915. They found that it required six times as much carbon tetrachlorid as it did of carbon bisulphid to kill the test insects. It was also reported on by Chittenden and Popenoe in 1911 and by Garman in 1913. McClintock, Hamilton, and Lowe reported in 1911 that it was one-fifth as insecticidal as sulphur dioxid, chloroform being somewhat more effective than carbon tetrachlorid and carbon bisulphid comparing more favorably with sulphur dioxid in insecticidal efficacy. Moore in 1917 published a study of volatility and toxicity in which he finds the volatility of carbon bisulphid, chloroform, and carbon tetrachlorid, in terms of gram molecules evaporating in 400 minutes, to be, respectively, 1.3616, 1.2870, and 0.7067, the carbon tetrachlorid being distinctly the least volatile; the respective toxicities, in terms of millionths of a gram molecule killing in 400 minutes, were 286.3, 894.6, and 161.9, carbon tetrachlorid being distinctly the least toxic. The test animal was the house fly. Very similar results in regard to the relative toxicity of these three compounds were published by Moore and Graham in 1918. During the war carbon tetrachlorid was noted as an effective insecticide for lice by Foster, Zucker, Galewsky, Alessandrini, and Hase. Dunn prefers it to chloroform for application to wounds, etc., infested with screw worm, but Bishopp finds it less satisfactory than chloroform.

PROPERTIES OF CARBON TETRACHLORID

In connection with its use as an anthelmintic, the points of interest covered in the literature noted are as follows: Carbon tetrachlorid is less volatile than chloroform or carbon bisulphid and is less toxic to insects than either; it does not seem to diminish the tone or contractility of unstriated musculature. Additional points in regard to this drug, in comparison with chloroform and carbon bisulphid, which have direct bearing on its anthelmintic action are as follows: Chloroform is soluble in 161 parts of water at 22° C., carbon bisulphid in 526 parts at 25°, and carbon tetrachlorid in 1,250 parts at 25°, carbon tetrachlorid being distinctly less soluble in water than either of the other substances. Carbon bisulphid boils at 46.25°, chloroform at 61.2°, and carbon tetrachlorid at 76.74°, carbon tetrachlorid boiling at a temperature 15° to 30°

higher than the other two substances. The relation of these facts to the use of carbon tetrachlorid as an anthelmintic are pointed out in a consideration of the anthelmintic studies in this paper. A final point to be noted is that pure carbon tetrachlorid must be used in testing. The drug may carry carbon bisulphid as an impurity, and Dr. Couch, of the Pathological Division of this bureau, tells me that phosgene, carbonyl chlorid, may be present under certain circumstances. This latter substance was used as one of the poison gases during the late war and has a marked depressant action on the heart.

ANTHELMINTIC EXPERIMENTS

In the experiments on dogs reported in this paper, the technic is the same as that given in previous papers by the writer alone or in collaboration with other investigators (*7–11*). The animals were given anthelmintic treatment, and all feces passed were collected once a day for 3 to 6 days, screened, and the worms passed were identified and counted up to the time of death. The animals were killed by illuminating gas, chloroform, or shooting through the head; and the worms present post mortem were identified and counted, the gross pathological conditions present post mortem being noted with especial reference to conditions due to the drug administered, allowance being made for the lesions due to the agent used in killing the animal. The protocols are abbreviated to the following form: Dog's number; dose in terms of mils (cubic centimeters) per kilogram, abbreviated to m. p. k.; worms passed daily, first day being the first day after treatment, that is, for the first 24 hours following treatment; post mortem, abbreviated to p. m. and showing the worms present; percentage of efficacy against kinds of worms present.

CARBON TETRACHLORID ALONE

In the following experiment carbon tetrachlorid was administered in capsules, followed immediately with 30 mils of castor oil, after the animal had fasted 18 hours.

Dog 367; 0.3 m. p. k.; no worms in 6 days; p. m., 7 whipworms; 0 per cent vs. whipworms.

In the following experiments carbon tetrachlorid was administered in capsules after the animal had fasted 18 to 24 hours, but without purgation.

Dog 381; 0.3 m. p. k.; 16 whipworms second day, 1 whipworm fourth day; p. m., 116 whipworms; 13 per cent vs. whipworms.

Dog 386; 0.3 m. p. k.; 6 ascarids first day; p. m., 1 whipworm, 15 *Dipylidium* sp.; 100 per cent vs. ascarids, 0 per cent vs. whipworm and *Dipylidium* sp.

Dog 387; 0.3 m. p. k.; 4 hookworms first day, 1 whipworm second day; p. m., 5 whipworms; 100 per cent vs. hookworms, 17 per cent vs. whipworms.

Dog 389; 0.3 m. p. k.; 25 hookworms first day; p. m., 8 whipworms; 100 per cent vs. hookworms, 0 per cent vs. whipworms.

Dog 390; 0.3 m. p. k.; no worms in 6 days; p. m., 50 whipworms, 10 *Dipylidium* sp.; 0 per cent vs. whipworms and *Dipylidium* sp.

Dog 392; 0.3 m. p. k.; 4 hookworms first day; p. m., 1 whipworm, 1 *Dipylidium* sp.; 100 per cent vs. hookworms, 0 per cent vs. whipworms and *Dipylidium* sp.

Dog 393; 0.3 m. p. k.; no worms in 4 days; p. m., scores of whipworms in cecum and upper colon, 45 *Diplyidium* sp.; 0 per cent vs. whipworms and *Dipylidium* sp.

Dog 394; 0.3 m. p. k.; 1 hookworm and 1 whipworm first day; p. m., 22 whipworms; 100 per cent vs. hookworms, 4 per cent vs. whipworms.

Dog 395; 1 m. p. k.; 16 whipworms first day; p. m., 1 whipworm; 94 per cent vs. whipworms.

Dog 396; 0.2 m. p. k.; no worms in 4 days; p. m., no worms; no conclusions.

Dog 397; 0.2 m. p. k.; 1 ascarid first day; p. m., 90 *Dipylidium* sp.; 100 per cent vs. ascarids, 0 per cent vs. *Dipylidium* sp.

Dog 398; 0.1 m. p. k.; no worms in 4 days; p. m., no worms; no conclusions.

Dog 399; 0.1 m. p. k.; 8 hookworms and 3 ascarids first day; p. m., 2 whipworms; 100 per cent vs. hookworms and ascarids, 0 per cent vs. whipworms.

Dog 401; 1.5 m. p. k.; no worms in 3 days; p. m., no worms; no conclusions.

Dog 402; 0.2 m. p. k.; no worms in 3 days; p. m., 3 hookworms, 2 ascarids, 1 whipworm; 0 per cent vs. hookworms, ascarids, and whipworms.

Dog 403; 0.2 m. p. k.; 1 hookworm first day; p. m., 9 whipworms, 1 *Dipylidium* sp.; 100 per cent vs. hookworms, 0 per cent vs. whipworms and *Dipylidium* sp.

Dog 404; 0.1 m. p. k.; 8 hookworms and 3 ascarids first day, 2 hookworms second day; p. m., 27 hookworms, 20 ascarids; 27 per cent vs. hookworms, 13 per cent vs. ascarids. (This dog also received 22 gr. of freshly ground moldy areca nut; it is a question whether this affected the result.)

Dog 406; 1 m. p. k.; 2 ascarids first day; p. m., 12 *Dipylidium* sp.; 100 per cent vs. ascarids, 0 per cent vs. *Dipylidium* sp.

Dog 407; 0.1 m. p. k.; 5 hookworms, 26 ascarids first day, 2 ascarids second day; p. m., 3 hookworms; 62.5 per cent vs. hookworms, 100 per cent vs. ascarids.

An examination of the findings for the 20 dogs listed above shows that in the 7 cases where dogs had Dipylidium the treatment with carbon tetrachlorid in doses from 0.2 to 1 m. p. k. was entirely ineffective against

this tapeworm, so the drug must be regarded as of no value against Dipylidium, and this will probably be true of tapeworms of other genera.

As regards the findings in the case of nematodes, they may be summarized as follows:

Carbon tetrachlorid, administered in capsules in dose rate of 0.1 m. p. k. to 4 dogs, removed 23 hookworms and left 30, an efficacy of 43 per cent; it removed 34 ascarids and left 9, an efficacy of 79 per cent; it removed no whipworms and left 2, an efficacy of 0 per cent.

Carbon tetrachlorid, administered in capsules in dose rate of 0.2 m. p. k. to 4 dogs, removed 1 hookworm and left 3, an efficacy of 25 per cent; it removed 1 ascarid and left 1, an efficacy of 50 per cent; it removed no whipworms and left 11, an efficacy of 0 per cent.

Carbon tetrachlorid, administered in capsules in dose rate of 0.3 m. p. k. to 9 dogs, removed 34 hookworms and left none, an efficacy of 100 per cent; it removed 6 ascarids and left none, an efficacy of 100 per cent; it removed 19 whipworms and left 210 plus scores uncounted in one animal, an efficacy of less than 10 per cent.

Carbon tetrachlorid, administered in capsules in dose rate of 1 m. p. k. to 1 dog, removed 2 ascarids and left 1, an efficacy of 67 per cent; it removed 16 whipworms and left none, an efficacy of 100 per cent; no hookworms were present in this dog.

Carbon tetrachlorid, administered in capsules in dose rate of 1.5 m. p. k. to 1 dog, removed no worms and none were found post mortem; no conclusions as to efficacy. The intent in this experiment was to ascertain whether any bad results would follow the administration of such a large dose. No bad effects of the treatment were observed ante mortem or post mortem.

It appears from the foregoing experiments that a dose rate of 0.3 m. p. k. is necessary to secure a dependable efficacy of carbon tetrachlorid against hookworms and ascarids. Whipworms, as the writer has pointed out in a number of papers, can not be removed with any certainty by single-dose treatment with any anthelmintic of which he is aware.

In the following experiments carbon tetrachlorid was administered by drench instead of in capsules to fasting dogs, without purgation.

Dog 409; 0.3 m. p. k.; 1 hookworm first day; p. m., 1 hookworm, 25 whipworms; 50 per cent vs. hookworms, 0 per cent vs. whipworms.

Dog 411; 0.3 m. p. k.; 8 hookworms and 25 ascarids first day, 2 ascarids third day; p. m., 16 whipworms; 100 per cent vs. hookworms and ascarids, 0 per cent vs. whipworms.

Dog 412; 0.3 m. p. k.; 7 hookworms and 16 ascarids first day; p. m., 21 hookworms, 22 ascarids, 54 whipworms; 25 per cent vs. hookworms, 40 per cent vs. ascarids, 0 per cent vs whipworms.

In the experiment described above carbon tetrachlorid, administered without capsules at a dose rate of 0.3 m. p. k. to 3 dogs, removed 16

hookworms and left 22, an efficacy of 42 per cent; it removed 43 ascarids and left 22, an efficacy of 66 per cent; it removed no whipworms and left 95, an efficacy of 0 per cent. By comparing these results with the results attained in administering carbon tetrachlorid at the same rate in capsules, it is evident that the change in mode of administration has been followed by a sharp decrease in efficacy from 100 per cent against hookworms and ascarids to 42 per cent against hookworms and 66 per cent against ascarids. We may conclude from this that the drug should be administered in capsules in order to develop its maximum effect.

In the following experiment the dog was given a large dose of carbon tetrachlorid and not killed, in order to ascertain whether there were any symptoms of delayed poisoning.

Dog 414; 1 m. p. k.; 1 hookworm first day; no post mortem and no conclusions as to efficacy. This dog was given 15 mils of castor oil and then 4 mils of carbon tetrachlorid, half of the entire dose of carbon tetrachlorid (weight of dog, 8 kgm.), without capsules. The dog staggered and appeared to be in a spasm. Another 15-mil dose of castor oil was then given, and another 4-mil dose of carbon tetrachlorid was given in capsules. The dog went down and stiffened out for a short time, then arose and staggered about as if intoxicated for a few minutes. The animal soon became normal in behavior and appearance and at the end of over four months has shown no evidence of bad effects. The transient toxic effects are to be attributed to the inhalation of the drug when given in large amount without capsules.

CARBON TETRACHLORID PLUS THYMOL

In order to ascertain whether thymol, which is readily soluble in carbon tetrachlorid, could be added to carbon tetrachlorid with any increase in anthelmintic efficacy, a solution was made up at the rate of 1 mil of carbon tetrachlorid to 10 gr. of thymol. This solution was administered in capsules in dose rate of 0.3 m. p. k. after fasting and without purgation as follows:

Dog 416; 5 hookworms and some *Dipylidium* sp. chains first day, some *Dipylidium* sp. chains sixth day; p. m., no worms; 100 per cent vs. hookworms and *Dipylidium* sp.

Dog 417; no worms in 4 days; p. m., 175 *Dipylidium* sp.; 0 per cent effective vs. *Dipylidium* sp.

Dog 418; 55 hookworms first day, 6 hookworms second day; p. m., 22 whipworms, 34 *Dipylidium* sp.; 100 per cent vs. hookworms, 0 per cent vs. whipworms and *Dipylidium* sp.

In the foregoing experiments this mixture, in the proportion of 1 mil of carbon tetrachlorid to 10 gr. of thymol, given in capsules at the rate of 0.3 m. p. k. to 3 dogs, removed 66 hookworms and left none, an efficacy of 100 per cent; it removed no whipworms and left 22, an efficacy of 0

per cent; no ascarids were present. The mixture appears to be as effective as carbon tetrachlorid alone in the same dose; a larger series of experiments might show that it was more effective.

CARBON TETRACHLORID PLUS CHENOPODIUM

In order to ascertain whether oil of chenopodium could be added to carbon tetrachlorid with any increase in anthelmintic efficacy, a solution was made up at the rate of 3 mils of carbon tetrachlorid to 1 mil of chenopodium. This solution was administered in capsules in dose rate of 0.3 m. p. k. after fasting and without purgation, as follows:

Dog 419; 1 hookworm and 2 whipworms first day, 109 hookworms, 8 ascarids, and 61 whipworms second day, 1 whipworm third day, 1 whipworm fourth day; p. m., 12 whipworms, 1 *Dipylidium* sp.; 100 per cent vs. hookworms and ascarids, 84.5 per cent vs. whipworms, 0 per cent vs. *Dipylidium* sp.

Dog 421; no worms in 8 days; p. m., 92 *Dipylidium* sp.; 0 per cent vs. *Dipylidium* sp.

It appears from the foregoing that a mixture of carbon tetrachlorid, 3 parts, and chenopodium, 1 part, given in capsules at the rate of 0.3 m. p. k. to 2 dogs, removed 110 hookworms and left none, an efficacy of 100 per cent; removed 8 ascarids and left none, an efficacy of 100 per cent; removed 65 whipworms and left 12, an efficacy of 84.5 per cent; removed no *Dipylidium* sp. and left 1, an efficacy of 0 per cent. The mixture is, therefore, as effective as carbon tetrachlorid alone in the same dose; a larger series of experiments might show that it was more effective.

In the following experiment, the above-mentioned mixture of carbon tetrachlorid and chenopodium was given at the same rate, 0.3 m. p. k., by means of a dose syringe to a dog that was too savage to dose successfully by means of capsules:

Dog 423; no worms in 8 days; p. m., 7 hookworms, 1 whipworm, 40 *Dipylidium* sp.; 0 per cent effective vs. hookworms, whipworms, and *Dipylidium* sp.

The complete failure of the treatment in this case, in contrast with the excellent results obtained in the case of dog 419, where the dose was given in capsules, must be attributed to the difference in the mode of administration. By the use of the dose syringe, part of the dose is lost in the syringe, part by evaporation from the mouth and by slobbering, and there may be other factors present that modify the result.

SUMMARY

An inspection of the results obtained in the foregoing experiments with carbon tetrachlorid shows that when given in capsules at a rate of 0.3 m. p. k. it constitutes a very effective treatment for hookworms in

dogs. It also shows a very high efficacy against ascarids. It removes whipworms when it enters the cecum, just as other anthelmintics do, but is substantially as uncertain in its action in single dose against this worm as are a number of other drugs. It is not of value in removing tapeworms of the genus Dipylidium and would probably be equally valueless against other tapeworms. It maintains its high efficacy against hookworms and ascarids when mixed with thymol at the rate of 1 mil of carbon tetrachlorid and 10 gr. of thymol, or when mixed with chenopodium in the proportion of carbon tetrachlorid, 3 parts, and chenopodium, 1 part, when given in capsules at a dose rate of 0.3 m. p. k. of these mixtures. The administration of the drugs in dosage less than 0.3 m. p. k. or without capsules is unsatisfactory. It should be noted in passing that in five of the cases noted where dogs were given from 0.1 to 0.3 m. p. k. of carbon tetrachlorid alone in capsules and no hookworms were found in the feces or post mortem, microscopic examination of the feces before treatment by the writer or by Messrs. Wigdor and Chapin of this laboratory had shown the presence of hookworm eggs. It is probable that in these cases the hookworms were removed by the drug but had not been found in the feces for one of two reasons. In the first place, in collecting feces from a cement floor 15 feet square, hookworms may be missed under certain conditions. In the second place, carbon tetrachlorid is one of the anthelmintics which frequently exert a destructive effect on the hookworm, half of the worm, usually the anterior half, being distorted or missing in worms collected from the feces. Caius and Mhaskar (*3*) note that some anthelmintics (as thymol) have this effect and others (as chenopodium) do not. Carbon tetrachlorid is more destructive to dog hookworms than any drug the writer has used on them.

RESULTS WITH OTHER ANTHELMINTICS

To ascertain just how effective carbon tetrachlorid is against hookworms in comparison with other drugs, the writer has summarized below the results published by him, alone or in collaboration with other workers, with other drugs against hookworms, noting other work of the same sort.

CHLOROFORM

Hall and Foster (*9*) found that chloroform in castor oil in dosage of 0.2 m. p. k. administered to 5 dogs removed 474 hookworms and left 356, an efficacy of 56 per cent.

Hall (*8, pt. III*) found that chloroform in dosage of 0.1 m. p. k. removed 0 hookworms from 1 dog and left 3, an efficacy of 0 per cent; in 0.2 m. p. k. to 2 dogs it removed 13 hookworms and left 76, an efficacy of 15 per cent; in 0.3 m. p. k. to 2 dogs it removed 3 hookworms and left 4, an efficacy of 43 per cent; in 2 m. p. k. it removed 1 hookworm and left 0,

an efficacy of 100 per cent; the total for the series of 6 infested dogs was 17 hookworms passed and 83 left, or 17 per cent for chloroform in dosage of 0.1 to 2 m. p. k. administered in castor oil. Chloroform administered in soft capsules in 15-minim doses on the first, fourth, sixth, and eighth days of an experiment removed no hookworms and left 6, an efficacy of 0 per cent; administered in soft capsules at the rate of 0.3 m. p. k. it removed no hookworms and left 4, an efficacy of 0 per cent.

The totals for all the foregoing tests with chloroform show that it removed 491 hookworms and left 445, an efficacy of 52 per cent.

CHENOPODIUM

Hall and Foster (*9*) found that chenopodium in doses of 0.3 m. p. k. in castor oil administered to 8 dogs removed about 25 per cent of their hookworms; in doses of 0.2 m. p. k. in capsules administered to 8 dogs daily for 3 successive days it was ineffective against hookworms.

Hall and Hamilton (*10*) found that the heavy fractions of oil of chenopodium, those distilling over at the higher range of temperature, administered in doses of 0.1 m. p. k. to 5 infested dogs removed no hookworms and left 68, an efficacy of 0 per cent; the light fractions administered in doses of 0.1 m. p. k. to 2 infested dogs removed 1 hookworm and left 76, an efficacy of little over 1 per cent.

Hall (*8, pt. I*), using chenopodium in repeated doses, found that in 2-minim doses daily for 18 days given to 3 infested dogs it removed 12 hookworms and left 9, an efficacy of 57 per cent; in 5-minim doses daily for 12 days given to 2 dogs it removed 1 hookworm and left none, an efficacy of 100 per cent; in 5-minim doses given 4 times in 1 week to 1 dog it removed 3 hookworms and left 11, an efficacy of 21 per cent; in doses of 0.5 m. p. k. daily on 2 successive days to 1 dog it removed 5 hookworms and left 16, an efficacy of 24 per cent; in 10-minim doses at 1-hour or ½-hour intervals for a total of 3 doses to 4 dogs it removed 10 hookworms and left 24, an efficacy of 30 per cent; in 5-minim doses at 1-hour intervals for a total of 3 doses it removed 1 hookworm and left none, an efficacy of 100 per cent. The totals show that, in repeated doses as given, chenopodium removed 32 hookworms and left 60, an efficacy of 35 per cent.

Hall and Wigdor (*11*), using the soft, or soluble elastic, capsules, found that chenopodium at the rate of 0.1 m. p. k. given to 7 infested dogs removed 23 hookworms and left 8, an efficacy of 74 per cent; using hard capsules instead of soft, chenopodium at the rate of 0.1 m. p. k. given to 2 infested dogs removed no hookworms and left 16, an efficacy of 0 per cent.

Hall (*8, pt. VI*), using enteric-coated capsules of chenopodium in single doses of 10 to 20 minims, found that it removed 4 hookworms and left 6, an efficacy of 40 per cent.

The totals for the foregoing tests with chenopodium or its constituents, so far as totals may be compiled, show that it removed 60 hookworms and left 234, an efficacy of 20 per cent.

CHLOROFORM AND CHENOPODIUM

Hall and Foster (*9*) found that chenopodium in doses of 0.1 m. p. k. and chloroform in doses of 1 minim per kilogram, given to 6 dogs daily for 6 successive days, removed 94 hookworms and left 39, an efficacy of 71 per cent; chenopodium and chloroform, at the rate of 0.1 m. p. k. each, given to 4 dogs in 1 dose removed 7 hookworms and left 54, an efficacy of 11 per cent; chenopodium and chloroform, at the rate of 0.2 m. p. k. each, was over 50 per cent effective against hookworms.

Hall (*8, pt. IV*) found that chenopodium at the rate of 0.1 m. p. k. and chloroform at the rate of 0.2 m. p. k., given to 15 dogs, removed 35 hookworms and left 4, an efficacy of 87.5 per cent; chenopodium at the rate of 0.05 m. p. k. and chloroform at the rate of 0.2 m. p. k., given to 3 dogs, removed 8 hookworms and left 2, an efficacy of 80 per cent; chenopodium and chloroform at the rate of 0.2 m. p. k. each, given to 4 dogs, removed no hookworms and left 1, an efficacy of 0 per cent; the totals for the cases in this paper in which single doses were used being 43 hookworms removed and 8 left, an efficacy of 84 per cent. In repeated doses, chenopodium in soft capsules, in 5- to 10-minim doses (at 1-hour intervals; not stated in paper) for 3 doses, each dose accompanied by ½ ounce of castor oil, followed by 4 mils of chloroform in ½ ounce of castor oil, given to 4 dogs, removed 127 hookworms and left 15, an efficacy of 89 per cent; chenopodium and chloroform in soft capsules, each containing 5 minims of chenopodium and 10 minims of chloroform, were given to 4 dogs in amounts of 1, 2, or 4 capsules and removed 8 hookworms and left 11, an efficacy of 42 per cent; these same capsules were given at the rate of 1 daily to 11 dogs, for 2, 3, 5, 6, and 7 days, and removed 45 hookworms and left 19, an efficacy of 70 per cent; the totals for the cases in this paper in which repeated doses were used were 180 hookworms removed and 45 left, an efficacy of 80 per cent.

Hall (*8, pt. VI*) found that enteric-coated capsules containing 5 minims of chenopodium and 10 minims of chloroform, given in a dose of 2 to 3 capsules, removed no hookworms and left 2, an efficacy of 0 per cent.

The totals for the foregoing tests of chenopodium plus chloroform, so far as totals may be compiled, show that it removed 324 hookworms and left 147, an efficacy of 69 per cent.

THYMOL

Hall and Foster (*9*) found that thymol in doses of 298 to 1,752 mgm., given to 9 dogs, removed 23 hookworms and left 128, an efficacy of 15 per cent.

Gaiger (*6*) gave thymol to 5 dogs in doses of 30 gr., repeated at 1-hour intervals for a total of 3 doses; the feces were not examined for worms passed, but the dogs were examined post mortem several days later and hookworms were found in 3 dogs. He then increased the dose to 40 gr. and gave this to 4 dogs, all of which had hookworms present post mortem.

SUMMARY

From the foregoing, the relative efficacy of carbon tetrachlorid and the other drugs commonly used to remove hookworms (chloroform, chenopodium, chloroform and chenopodium, and thymol) appear to be about as follows:

Carbon tetrachlorid in less than the ascertained effective dosage (0.3 m. p. k.) or improperly administered (without capsules) removed 40 hookworms and left 55, an efficacy of only 42 per cent; but in the effective dosage of 0.3 m. p. k. in capsules it removed all the hookworms from 9 dogs and, judging from the fecal examinations, apparently removed all the hookworms from 5 other dogs, an efficacy of 100 per cent. A solution of thymol in carbon tetrachlorid in capsules was likewise 100 per cent effective against hookworms in the 2 infested dogs to which it was given. A solution of chenopodium in carbon tetrachlorid in capsules was likewise 100 per cent effective against hookworms in the one infested dog to which it was given.

On the other hand, chloroform in doses of 0.1 to 2 m. p. k. in castor oil was only 54 per cent effective against hookworms; in single or repeated doses in soft capsules it was entirely ineffective.

Chenopodium and its constituents in single or repeated doses have an average efficacy of 20 per cent against hookworms; in the dose commonly employed for removing hookworms from man, three 10-minim doses at 1-hour intervals, it is only 30 per cent effective, and its maximum efficacy in any series of dogs used is only 74 per cent.

Chloroform and chenopodium for all cases reported shows an average efficacy of 69 per cent, the maximum efficacy for a series being 89 per cent in cases where dogs were given three 5- to 10-minim doses in soft capsules at 1-hour intervals, each dose accompanied by ½ ounce of castor oil and followed a half hour later by 4 mils of chloroform in ½ ounce of castor oil, this dose of chloroform being in excess of 0.3 m. p. k. for the average dog (a 10-kgm. dog).

Thymol shows an efficacy of 15 per cent and according to Gaiger's record left hookworms in 7 of 9 dogs treated, with no evidence as to infestation on the part of the other 2 dogs.

Nothing heretofore reported for experimental tests of anthelmintics on dogs shows the 100 per cent efficacy for a series of tests against hookworms that carbon tetrachlorid does. The best results heretofore have been obtained by repeated doses of chenopodium followed by chloroform.

Chloroform alone appears to be better than chenopodium alone, and thymol makes a very poor showing.

ANTE-MORTEM AND POST-MORTEM CONDITIONS

In recommending that a drug be used as an anthelmintic, the question of safety must be given as much consideration as that of efficacy. As regards the treatment of dogs, the writer has administered carbon tetrachlorid to 30 dogs in doses of 0.1 to 1.5 m. p. k., the latter dose being five times the dose required to obtain dependable efficacy against hookworms, and has observed symptoms of any disturbance in only 1 dog, in which case the drug was given at the rate of 1 m. p. k., half of this dose being given without capsules. In this case the prompt evidence of intoxication was obviously due to inhalation, and the effect was very transient, disappearing in a few minutes. This dog was not killed and is alive 4 months later with no evidence of delayed poisoning of any sort.

Of the 29 dogs examined post mortem, 14 were killed with illuminating gas, 9 with chloroform, and 6 by shooting. It seems advisable in experiments of this sort to use different methods of killing, as the method used modifies the post-mortem picture, a fact which the writer (7) has already emphasized. The methods used here require that the post-mortem findings be made with the following facts in mind: Illuminating gas produces the characteristic picture of carbon-monoxid poisoning, with the peculiar pink color of the blood and with this pink color present over a large part of the digestive mucosa. Chloroform produces a congestive condition of the digestive tract, of the liver and kidneys, and of other organs. Shooting through the head produces characteristic lesions in the form of hemorrhages in the heart or lungs or both, and where the hemorrhage from the bullet wound is severe, the organs, especially the liver and kidneys, are light-colored from loss of blood. These dogs were shot with a .38-caliber revolver, causing profuse bleeding. As previously noted (7), a .22-caliber revolver, properly used, is large enough.

With the foregoing facts in mind, the 29 dogs were examined and the following conditions found: The heart was normal. The lungs, aside from a negligible anthracosis in some cases, showed a few apparent ecchymoses in 2 dogs killed with illuminating gas, and some areas of apparent local inflammation in 3 dogs killed with illuminating gas and in 2 dogs killed with chloroform. The liver was apparently normal macroscopically and showed nothing resembling the acute yellow necrosis produced by chloroform in doses of 0.3 m. p. k. or less. The spleen was normal or showed in 1 or 2 cases conditions not related to the carbon tetrachlorid. The kidneys showed the pathological conditions which are almost always present in dogs, but nothing that could be attributed to the carbon tetrachlorid. The bladder showed petechiae in 2 cases—not altogether rare in dogs—and seemed somewhat congested where the mixtures of thymol or chenopodium were used. The stomach was catarrhal

in 5 cases and showed some evidence of hemorrhage in 1 case. The small intestine showed some evidence of apparent local inflammation or slight hemorrhage in 7 cases, but this number of cases is so small compared to the number of animals used that it hardly warrants the belief that the drug was responsible for the condition. The large intestine was normal. The cecum showed hyperplastic glands in some cases, not an uncommon thing, and in 3 cases there were small inflamed areas not in relation to whipworms present. A low-grade inflammation at the site of whipworm attachment, usually at the tip of the cecum, is common in dogs, as a similar condition is in man.

In the writer's experience the foregoing findings suggest that carbon tetrachlorid will prove to be safer for use against hookworms in the dog than chloroform or chenopodium, as well as more effective than either. It is thought that the same may prove true in the treatment of man. The reason why it is perhaps safer as well as more effective than chloroform may be found to some extent from a reconsideration of its physical properties. As already noted, carbon tetrachlorid is distinctly less volatile than chloroform and has a higher boiling point, is much less soluble in water, does not diminish the tone of unstriated musculature, and, so far as tests on insects have any bearing on its effects on the higher animals, it is less toxic. It may be supposed, then, that carbon tetrachlorid will evaporate less rapidly in the stomach and intestines, allowing the drug to go farther with less absorption; its lesser solubility in water will also retard its absorption; the fact that it is not depressant to unstriated musculature will permit of the maintenance of peristalsis and the passage of the drug along the digestive tract; and its apparent lesser toxicity makes it safer to the host. To substantiate part of these statements, it may be noted that the drug has been found entirely safe in the absence of any purgation, and, macroscopically it has shown nothing comparable to the acute yellow necrosis of the liver produced by chloroform. Two dogs receiving 0.3 m. p. k. and 1 dog receiving 0.1 m. p. k. passed no feces in the first 24 hours after dosing, and these animals showed no evidence of toxic effects. That the drug is not constipating is shown by the fact that the 3 dogs receiving 1 m. p. k. and the dog receiving 1.5 m. p. k all passed feces within 24 hours after treatment.

CONCLUSIONS

So far as may be judged from experiments on 30 dogs, carbon tetrachlorid in doses of 0.3 m. p. k., administered in capsules, is more effective against hookworms in the dog than any of the drugs commonly used to remove hookworms, even when these are used in such combinations as chloroform and chenopodium. It appears to be a very safe drug, giving rise to no evident symptoms or post-mortem lesions even in doses five times as large as are necessary to give dependable efficacy against hookworms. It is cheap and deserves to be tested in human and veterinary

medicine. Because of the possibility of impurities, only a pure and carefully refined carbon tetrachlorid should be used. While this drug deserves consideration with especial reference to hookworms, it is also very effective for removing ascarids, being somewhat inferior to chenopodium in this respect, and will remove whipworms, as other anthelmintics will, when it gets into the cecum. It is not of value against tapeworms.

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